

Summit gets something done

Contrary to most accounts, last week's summit meeting in London was not a waste of time, but an occasion when several national leaders made promises they can now be asked to keep.

IN the past week, since seven of the eight participants in the London summit meeting packed their bags and went home, it has been commonplace for observers of the scene to say that the meeting accomplished nothing, and that there should be a different format for future meetings, which nevertheless are unlikely to serve much purpose either. But that is far from the truth. Did not the summit at least inform the population of one of the largest cities in the world that "G7" consists of a group of politicians so important that they have a licence to cause traffic chaos by travelling everywhere with motorcycle outriders or, otherwise, by helicopter? The slight confusion, that on this occasion there appeared to be eight members of the club (counting Mr Mikhail Gorbachev from the Soviet Union) is unlikely to recur; the founding members, who were the seven most prosperous members of the Organization for Economic Cooperation and Development (OECD) in 1975, were evidently embarrassed that, having politely refused Gorbachev's plea for financial help in advance of the meeting, their consolation prize seemed paper-thin.

But what happened last week is important in several respects. Most immediately, the members of G7 have committed themselves to "an effective framework convention on climate change" in time for next June's international conference in Brazil. True, the US delegation disappointed those clamouring for a treaty with numbers, but that is no surprise (and is also wise). Their disappointment should not conceal from them the simple truth that the G7 statement includes a commitment by the United States that there should be a treaty of some kind, together with a promise to work for "protocols to reinforce the treaty". Who, at this stage, could reasonably ask for more?

The seven heads of government also committed themselves to completing the current round of negotiations with the General Agreement on Tariffs and Trade (GATT) by the end of a year, or just 55 weeks behind schedule. Failure could lead to the impoverishment of rich and poor countries alike. The British prime minister has apparently been given power to call a further meeting if, as is probable, the promises are not being kept. The stumbling block, this year as last, will be European agriculture, whose waywardness on internal and external trade impoverishes both Europe and the rest of the world.

The European Communities' sins on agricultural

policy are notorious. The objective (specified in the Treaty of Rome) is that farmers should not be disadvantaged by the rapid industrial growth foreseen at the outset. The result has been a dual system of support for farmers. Market prices are kept artificially high by import levies and by an undertaking that the European Commission will take surplus food off the market at fixed prices. Europe's taxpayers, as consumers of food, meet the cost of needlessly high prices and the cost of the more direct subsidy of farmers through the taxes they pay. What maddens the rest of the world is that Europe's farmers are protected from the competition of low-cost farmers elsewhere. To add insult to injury, Europe unfairly competes with overseas producers by dumping its surpluses on whatever customers it can find.

There is now talk of reforming this system, chiefly by reducing guaranteed prices and by requiring farmers to take 15 per cent of their land out of production; smaller producers will be fully compensated for doing so, larger producers less so. The numbers being canvassed will increase the burden on Europeans by more than the cost to them as consumers will be reduced, but the end result will be that a less efficient European farming system will still be intolerably subsidized when seen from outside. The chances are that Mr John Major will have to hold his emergency meeting; he will not have an easy time, given that his own farmers (who are a rumbustious lot) can be an important influence in what must be an election year. But at least it is something that the heads of government have committed themselves to succeed.

A further achievement (strictly speaking, outside the summit proper) is the deal struck between the United States and the Soviet Union on the long-distance negotiations on strategic missiles, which have been under way for 15 years. In the event, both powers have agreed to reduce their stocks of usable nuclear warheads by rather less than a half, have set aside worries about anti-missile missile defences (perhaps in the hope that neither can afford them) and have settled on rules for the development of new missiles. So why only a 40 or so per cent reduction of the capacity of one side to annihilate the other? That is what the sceptics ask. Their answer is that the proposed treaty (to be signed in Moscow later in the month) gives each side the right to inspection of the other's weapons. That could be crucial when the question of who is literally in charge of Soviet weapons may keep people in the West awake at nights.



The other important issue is one on which G7 failed to reach an imaginative solution: what should be done to help the Soviet Union through the next few years? Gorbachev would probably not have refused a latter-day equivalent of the post-Second-World-War Marshall Plan, but had been warned that there would not be such a plan on offer. G7 is probably correct in its view that, while the Soviet economy remains hamstrung by artificial prices and the affectation of central planning (which seems mostly to have broken down), as well as by uncertainty about the relationship between the centre and the republics, mere money might be wasted. But the proposed special (and less than full) membership of international institutions such as the International Monetary Fund is altogether inadequate.

The Soviet Union's difficulties are not a consequence of ignorance in high places in Moscow about the nature of a market economy; the people are as literate there as anywhere — perhaps more so — and, in any case, institutions in the West are forever arranging seminars on the subject on their behalf. The difficulty, rather, is ordinary people's resentment (after 70 years) of what the transition to such a system would entail. So much should be clear from the way in which the cooperative organizations, first allowed four years ago, are being gradually strangled by legislation born of people's unwillingness to pay more than controlled prices even for goods not otherwise available. (The chief result is that the black market grows instead.)

It is more understandable that many of those performing pointless jobs, or jobs that cannot be afforded at a time of economic collapse (including much of the huge scientific establishment), should fiercely resist the abolition of them (especially because the Soviet Union has only a token system of social security). And there is bound to be fierce resentment, politically expressed, of the general impoverishment begun and now in prospect. Yet with industrial output down by 17 per cent in a year (a much greater fall than in the United States during the Great Depression), no amount of money from the West could make good the difference.

Yet what the Soviet Union needs is an *in situ* demonstration of success, and a tangible proof that economic success is not inimical to civility (but the opposite). The belief that there may be something different about the Soviet Union is not just superstition — there were, after all, those 70 years. But the Soviet people now resisting change would benefit enormously if they were shown that market principles can work, and create wealth, even in the Soviet Union.

In this spirit, China has benefited greatly — and not just materially — from the example of the “new economic zones” established eight years ago. Did nobody think of suggesting something on these lines to Gorbachev, on the understanding that it should be possible to arrange on a narrow geographical basis to remove the present inhibition of voluntary capital investment in the Soviet Union — the risk that inward investment will be mismanaged and the likelihood that it

will never be recoverable? Is not that a project that would have spanned the West's understandable caution and Gorbachev's need to get something moving? □

Milk and coffee

The British Anglican plan to boycott Nestlé coffee leaves much logic by the wayside.

RELIGIOUS people revel in sharpening contradictions, which is the most charitable explanation why the body called the Synod of the Church of England decided last week to appeal to Britain's Anglican community to boycott the coffee widely sold in Britain (and elsewhere) by the Swiss-based company called Nestlé. The essence of the case is that Nestlé continues to sell dried milk supplemented by vitamins for infant feeding (otherwise known as “formula”) in many of the poor countries of the world, that many of the mothers there thus deprive their children of the even more nutritious food they themselves secrete naturally, that in the process badly sterilized feeding bottles and access ports (known as “nipples”) become a source of bacterial infection and that, as a consequence, the use of infant formula, far from beneficial, actively causes harm. Not buying Nestlé's coffee, the Synod seems to argue, will make the company see the error of its ways with infant milk.

Sadly, the issue is not as simple as the churchmen think. There is little doubt that the benefits of infant formula have often been oversold in poor countries, which is why the World Health Organization has promulgated a code of practice demanding that they should advertise the principle that “mothers' milk is usually best”. In principle, that should ensure that manufacturers will no longer play on the credulity of unsophisticated mothers in developing countries, and in particular on the suspicion that Western proclivities for bottle feeding (now on the wane) explain the West's success. There are also restrictions on the use of free samples of infant formula; it has too often been difficult to tell which were meant for promotional and which for compassionate reasons. The error in the Synod's conclusion last week is the implied assertion that a freedom many mothers in industrialized countries value should be denied to their less fortunate counterparts (or “sisters” as they are often known) elsewhere.

Indeed, the paradox has endless ramifications. Mothers' milk is certainly best, but what is to happen if a particular mother does not have enough of it? Or if she carries an infection, say by the AIDS virus.

It would be a great surprise if the Anglican community in Britain could secure a majority among its own members for self-imposed restrictions of the kind that it is advocating for others. Yet, to be logical, it should try. Otherwise, it runs the risk of seeming neo-colonialist or even — more dangerous — anti-feminist. Or is it that the Synod harbours a secret agenda, and that its real animus is directed against coffee-drinkers? □

German research budget robs west to pay east

- Funding state-of-the-art institutes in east
- National labs, in west, are facing cuts

Munich

EARLIER this month, Heinz Riesenhuber, the German Research Minister, announced a generous increase in support for research in the former East Germany, thereby completing the foundation for a competitive research infrastructure in the east. But to finance the increase for the east, Riesenhuber had to slash the budgets of research institutions in the west for 1992 and future years, hitting Germany's 13 national laboratories with cuts of up to 30 per cent over the next three years.

Now, western German researchers are afraid that the shift of funds from west to east will turn out to be a shortsighted manoeuvre that, paradoxically, could hurt eastern as well as western Germany.

Riesenhuber's new 1992 budget gives the research ministry DM9,252 million (about \$5,200 million), which represents a 9.7 per cent increase over the 1991 budget — not very much in light of the fact that Germany's population has increased by more than 25 per cent through unification.

The new budget has plenty of good news for research in the east, however. Riesenhuber committed a total of DM1,600 million to research in eastern Germany in 1992, including financing both for new non-university research institutions and for projects at universities and in industry. Over the next three years, he said, he expected to commit more than DM6,000 million to research in eastern Germany.

The research ministry is planning to establish at least three new national laboratories in the east — an environment research institute in Halle, a biomedical and clinical research unit in Berlin-Buch, and a newly announced centre for geosciences in Potsdam.

In addition, Riesenhuber said he was in general agreement with recommendations by the science advisory council, the Wissenschaftsrat, to establish Max Planck institutes or research groups in several places in eastern Germany, the latest recommendation being to set up an institute for colloid chemistry and surface science in Berlin-Treptow. Wissenschaftsrat spokesman Wilhelm Krull said it was especially encouraging that the research ministry had been so generous with support for researchers at the new institutions. The average level of ministry support for a researcher at one of the institutions in the east will be DM138,000 a year including salary. Given the low

salary levels in the east, said Krull, this will allow the purchase of significant amounts of new equipment in the first few years.

So much money is being pumped into research in the east, said Krull, that he expects some of the institutes there to be doing state-of-the-art work, especially in applied research, in just three to four years. "Industry is already queueing up" to offer contracts for research at some institutes, he said, and this will be the case at more and more institutes as the new equipment is installed.

Western Germany does not do nearly so well in the 1992 budget. Particularly hard hit are the 13 existing national laboratories, all located in the west. Riesenhuber froze the budget for these laboratories at its 1991 level for the next three years, forcing the laboratories to make cuts equivalent to the rate of inflation, which is expected to be 3–4 per cent a year, or roughly 11 per cent over the next three years.

As the laboratories depend on the ministry for 90 per cent of their support these cuts will hurt, but there is more bad news: Riesenhuber also cut back

development of so-called 'key technologies' such as microchips and high-tech aircraft, the cuts represent a "paradigm shift" in the funding philosophy of the ministry, said Peter Silberbach, an official at the Science Ministry of the *Land* of North Rhine-Westphalia.

Bonn Research Ministry spokesman Walter Mönig denied that the cuts represented anything so ominous or sudden as a paradigm shift. Such reductions have been going on for years, he said.

The Bonn region, and North Rhine-Westphalia in general, stands to be a big loser if the cuts are carried out in as harsh a form as is planned. In addition to the mathematics and data processing society, the German Aerospace Research Establishment in Cologne-Porz near Bonn also stands to lose badly.

Many in Germany are worried about how the new budget could compromise Germany's competitiveness. Hubert Markl, president of the German funding agency Deutsche Forschungsgemeinschaft, has argued that the least effective way to alleviate the "neediness and suffering" of eastern Germany is to cut back on investment in research and development in the west. "Our international competitors are not going to reduce their investment in R&D just because Germany needs to take a unity-induced breather," Markl said.

In at least one sense, making cuts in national laboratories might seem a blessing in disguise, given that the laboratories have long been criticized for being over-

- (1) AWI: Alfred Wegener Institute for Polar and Ocean Research, Bremerhaven
- (2) DESY: Germany Electron Synchrotron, Hamburg
- (3) DKFZ: German Cancer Research Centre, Heidelberg
- (4) DLR: German Aerospace Research Institution, Cologne
- (5) GBF: Society for Biotechnological Research, Braunschweig
- (6) GKSS: Research Centre Geesthacht, Geesthacht
- (7) GMD: Society for Mathematics and Data Processing, Bonn
- (8) GSF: Centre for Environment and Health Research, Neuherberg
- (9) GSI: Society for Heavy-Ion Research, Darmstadt
- (10) HMI: Hahn-Meitner Institute, Berlin
- (11) IPP: Max Planck Institute for Plasma Physics, Garching
- (12) KFA: Research Centre Jülich, Jülich
- (13) KFK: Nuclear Research Centre Karlsruhe, Karlsruhe



Germany's National Labs, mainly in the former West Germany, will bear the brunt of cuts.

drastically on the amount of 'soft' money available for project orientated research, most of which flows to national laboratories and universities. As a result, certain laboratories, such as the Society for Mathematics and Data Processing in Bonn, are facing cuts of up to 30 per cent by the end of 1994.

Because some of the project money from the ministry had been used for the

staffed and inflexible. But Krull of Wissenschaftsrat and others warned that, unless the ministry takes the utmost care in setting priorities — and allows the laboratories to dismiss people who have lifetime contracts — then these cuts could hurt the projects, particularly the newer ones, which are most likely to be internationally competitive.

Steven Dickman

Mayo new head at Bell Labs

Washington

WHEN John Mayo was named the new president of AT&T Bell Laboratories early this month, he took over one of the world's most famous industrial research laboratories at a pivotal time in its history. In the past year and a half, AT&T has been shifting the emphasis at Bell Labs towards a more applications-orientated approach to research, and a number of researchers have left the laboratories amid charges that the days of free-wheeling, anything-goes exploration are rapidly disappearing.

Mayo, who is 61, seems to have been chosen to move the laboratories into this new role. He himself has moved up through the ranks at Bell Labs over the past 36 years while doing research that lay at the core of AT&T's business innovations — he worked on the first transistorized digital computer and on digitalizing telecommunications networks, he was a part of the Telstar satellite programme and he has been involved in developing integrated circuits and photonic devices.

"There is a mainstream to the technology" that AT&T is interested in, Mayo says, and his goal is "a nurturing of research to that mainstream". He insists that he does not want to do anything that will diminish the laboratories' strength in basic research, but researchers there are likely to find their environment changing noticeably over the next few years.

"The principal thing I would want to change is to steadily increase the flow of innovations to the marketplace," Mayo

says. To do this, he plans to emphasize teamwork, where teams will include everyone from basic researchers to marketing personnel. He foresees "more teams where good basic research is being done as part of a concept or a product or service". This, he says, should make researchers' jobs more fun because they will see their research applied more quickly.



Mayo: eyes on the marketplace

Making research sensitive to market needs will not necessarily squash the creativity and open inquiry that have been Bell Labs' trademarks for decades, Mayo says; indeed, such a sensitivity can stimulate basic research. "When the marketplace became discouraged with vacuum tubes, we got lots of research into materials — it came from the perception of a need," he says. The result was the transistor.

Mayo adds that Bell Labs will still have a place for basic research that has no direct connection with AT&T products. "We're not diminishing that." Nonetheless, it seems likely that by the time Mayo reaches retirement in a few years, Bell Labs will be a much different place from what it once was.

Robert Pool

National Institutes of Health Hadley quits two OSI investigations

Washington

IN the latest twist in the troubled history of the US National Institutes of Health (NIH)'s Office of Scientific Integrity (OSI), Suzanne Hadley, the OSI's former deputy director, has resigned from the office's two most controversial investigations. Hadley left the OSI in March to take up a post in NIH's Science Policy and Legislation Division. But to provide some continuity, she continued to handle the OSI investigations into the scientific fraud allegedly perpetrated by Tufts University immunologist Thereza Imanishi-Kari, and the disputed claim of National Cancer Institute researcher Robert Gallo to have co-discovered the AIDS virus.

But Hadley resigned from both these investigations on 1 July. "I resigned from the cases because I believed I could no longer be effective and because I no longer had full support [from NIH]," she says. She declines to comment any further, but NIH director Bernadine Healy is widely reported to be annoyed by the editorial

tone of a widely leaked draft of the OSI's report on the Imanishi-Kari affair, which painted whistleblower Margot O'Toole as the heroine of the case.

The departure of Hadley is likely to bring about Healy's first serious brush with Representative John Dingell (Democrat, Michigan), the formidable congressional watchdog of NIH. Staff at Dingell's House subcommittee on oversight and investigations interviewed Healy, NIH deputy director William Raub and Hadley last week, and have pencilled in a hearing for 31 July to examine Hadley's resignation from the two investigations. Dingell's staff are concerned that Hadley's departure, and with it her knowledge of the history of the two cases, could undermine the OSI's final reports. Although the Imanishi-Kari investigation is almost complete, the key chapter of the Gallo report that includes the results of biological tests on the identity of viral samples central to the case has yet to be written.

Peter Aldhous

NIH quick off the mark

Washington

THE US National Institutes of Health (NIH) last week took the unusual step of granting Barr Laboratories a non-exclusive patent licence to market a generic form of AZT, the only fully approved drug for the treatment of AIDS. The move struck some as premature because at this point NIH have no patent rights to AZT, and thus the licence is contingent upon a court deciding that NIH deserve a share of the six AZT-related patents now owned by Burroughs Wellcome.

Barr Laboratories, a generic drug company based in Pomona, New York, is now embroiled in a patent dispute with Burroughs Wellcome of North Carolina. Barr asserts that much of the research that demonstrated AZT's effectiveness against human immunodeficiency virus (HIV) — which in turn led to Burroughs Wellcome being awarded six patents for the use of AZT in the treatment of AIDS in 1988 — was in fact undertaken by researchers at the National Cancer Institute (NCI) and Duke University. Barr contends, therefore, that Wellcome has unfairly reaped the commercial benefits for a drug developed using taxpayer's money (*Nature* 351, 508; 1991.)

For its part, Wellcome argues that the NIH are trying to rewrite the rules of the cooperative research agreement that the company entered into with NCI in March 1985 and calls the NIH move "unprecedented". Such actions, the company maintains, could have a chilling effect on research collaborations.

NIH spokesman Don Ralbozski replies that this case over inventorship rights to AZT is likely to be "one of a kind" and says he hopes it will not "impair future collaborations with the private sector", which the NIH are keen to promote.

Even with the NIH licence in hand, Barr has a long way to go to sell a generic form of AZT in the United States. It must first win the court case against Wellcome and then obtain marketing approval by the US Food and Drug Administration for its version of AZT. And if all that goes well, Barr still cannot sell the drug until next July, when Wellcome's rights to sell AZT as an orphan drug run out. (Under the Orphan Drug Act of 1983, companies that develop drugs for the treatment of diseases that affect fewer than 200,000 people receive certain advantages.)

Opening up the market to generic competition could cut the price of AZT — which costs patients between \$2,200 and \$2,800 a year — by as much as half, Barr estimates.

Diane Gershon

Zagury report censures NIH

Washington

THE US National Institutes of Health (NIH) failed to ensure the safety of human subjects in AIDS vaccine and immunotherapy trials that were led by the controversial French AIDS researcher Daniel Zagury and involved researchers from the institutes' Bethesda campus. That is the conclusion of a report released last week by the NIH Office for Protection from Research Risks (OPRR).

The report found a "disjointed, compartmentalized system" at NIH for protecting human subjects, and it gives NIH until mid-September to put matters right.

The OPRR investigation was launched in July last year, after journalist John Crewdson of the *Chicago Tribune* alleged that clinical trials involving NIH researchers and carried out in Zaïre and France since 1986 had breached US regulations. The projects examined by OPRR include the much-publicized first human trials of a putative AIDS vaccine, which were carried out in Zaïre and reported in *Nature* (326, 249; 1987 & 332, 728; 1988).

OPRR's findings make disturbing reading. NIH failed to make their researchers aware of US Department of Health and Human Services regulations on the use of

human subjects in clinical trials, the report claims. Researchers collaborating with Zagury were unaware that these rules applied to research carried out abroad as well as in the United States. Consequently, NIH researchers sought the required approval for their research from a review board at their own institutes in only a few of the nine projects examined in the report.

Zagury disputes the OPRR contention that NIH researchers were in fact true collaborators on his projects and thus needed NIH approval (see *Nature* 352, 180; 18 July 1991), and his lawyer has written to OPRR director Charles McCarthy describing the report as "eminently libelous".

In their official response to OPRR, NIH say that a new Office of Human Subjects Research will in future oversee all research with human subjects, and educate all NIH researchers about relevant regulations. Until earlier this year, this was the responsibility of Saul Rosen, acting director of NIH's Warren Grant Magnuson Clinical Center. OPRR's investigation found that Rosen exerted little authority over NIH researchers outside the clinical centre itself.

Although OPRR reserves its strongest criticism for NIH, the report also singles out Zagury and National Cancer Institute researcher Robert Gallo (who is listed as co-author on 14 of Zagury's publications involving research with human subjects) for their "continuing lack of understanding about Health and Human Services human subjects regulations".

When the Zaïrean vaccine trials were first reported in the media, Zagury was portrayed as a hero because he had also injected the vaccine into himself. Only later did it emerge that the "small group of Zaïreans" described in the first *Nature* paper were children.

The Zaïrean trials relied heavily on technical expertise and materials provided by NIH researchers. In particular, the OPRR report notes that Zaïrean subjects were initially injected with a recombinant vaccinia virus, containing genetic material from human immunodeficiency virus, supplied by National Institute of Allergy and Infectious Diseases researcher Bernard Moss for use in animal research.

The OPRR is reserving its final word on the Zagury affair until it has more information on the extent of any harm suffered by the subjects involved in the various experiments. Three French AIDS patients and one Zaïrean enrolled in immunotherapy trials using the recombinant vaccinia are known to have died, but Zagury maintains that the Zaïrean children involved in the vaccine trials remain healthy.

Peter Aldhous

DDI nears approval

Washington

DIDEOXYINOSINE (DDI) should soon become the second antiviral drug approved by the US Food and Drug Administration (FDA) for use against human immunodeficiency virus (HIV). The Antiviral Drug Products Advisory Committee, an independent panel that advises the FDA, decided last Friday to recommend approval for the drug to treat AIDS patients not responding to treatment with zidovudine (AZT). The FDA is expected to endorse the recommendation.

The advisory committee's 5:2 vote in favour of approval was welcomed by many AIDS activists, who have long argued that the US drug approval process should be streamlined, to counter the AIDS epidemic. That message seems to have been heard by the antiviral advisory committee, which recommended approval for DDI, despite the fact that the data presented by its manufacturer, Bristol-Myers Squibb, on the efficacy of the drug in fighting AIDS, were far from complete.

Like Wellcome's AZT, DDI slows replication of HIV by inhibiting the enzyme reverse transcriptase. But up to 40 per cent of patients have to come off AZT after a year of treatment, because of side effects — including nausea and muscle wasting. Viral resistance to AZT can also occur.

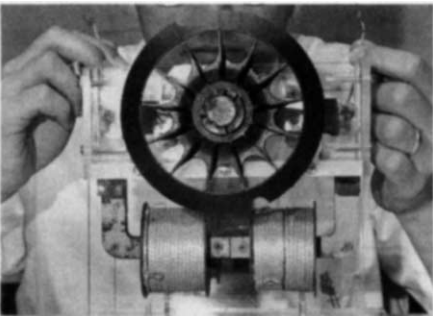
A third reverse transcriptase inhibitor, Hoffmann-La Roche's DDC, is also awaiting FDA approval as an anti-HIV agent. But once this drug is approved, no new AIDS drugs will be approved for several years, unless the current process is reformed.

This time could be reduced, however, by a new proposal for 'accelerated' drug approval, currently being drafted by FDA officials. The new class of approval is expected to apply not only to AIDS drugs, but also to experimental drugs developed to treat other life-threatening or seriously debilitating diseases such as cancer or Alzheimer's. John Petricani, vice-president of the Pharmaceutical Manufacturer's Association, says the new proposal, to be announced later this summer, is expected to allow approval without conclusive proof that a drug can extend life, provided that further clinical studies are carried out after the drug is first marketed — in essence, formalizing the process that has happened for DDI.

The AIDS drugs that may be affected by the FDA's fledgling proposal include BI-RG-587, from Boehringer Ingelheim, and Merck's L-drug. Unlike AZT, DDI and DDC, which were all developed for other purposes before finding a second lease of life as AIDS drugs, this next generation of anti-HIV agents were designed specifically to counter HIV infection.

Peter Aldhous

Superconducting motor



COILS of a bismuth-based superconductor form the heart of this 25-watt, 0.5-amp motor which powers the small fan above it. "To our knowledge, this is the first time anyone has built a superconducting motor using high-temperature superconducting coils that produces a usable power output," says Gregory Yurek, president of American Superconductor Corporation of Watertown, Massachusetts, which built the coils. Reliance Electric of Cleveland built the motor. Superconducting motors are one of the most potentially important applications of high-temperature superconductors because large motors for industrial and commercial use account for a major portion of all electric power consumption, and even a small increase in motor efficiency could save a lot of money.

R.P.

Teething troubles for UK technology labs

London

A THREE-YEAR-OLD experiment in government-industry research cooperation in Britain — set up in parallel to similar US efforts — has run into trouble and appears to be heading for a shake-up. The first two of the programme's 12 Interdisciplinary Research Centres (IRCs) have received critical reviews by independent experts, and, in a climate of funding cutbacks, at least one of them could eventually be closed, sources say.

The poor performance of the first centres to be reviewed is an inauspicious beginning for the ambitious UK effort, and a worrying harbinger of things to come. Intended as Britain's answer to a recent series of US government-industry laboratories (the Science and Technology Centers and Engineering Research Centers of the National Science Foundation), the IRCs are remarkably similar to their US counterparts. Both countries give their centres four-year rolling grants (averaging about £2 million annually for each of the UK centres) and expected them to match the government funding with support from industrial and academic co-sponsors, largely to encourage transfer of technology between the sectors.

But the UK programme had the misfortune of coming just as the British economy was about to hit a downturn and

the budget of its sponsor, the Science and Engineering Research Council (SERC), was facing a £40 million shortfall. Economics alone cannot, however, explain the problems of the University of Cambridge's centre for superconductivity and the centre on engineering design run by a consortium headed by the University of Glasgow, the two centres now under SERC review. As the independent researchers who examined the two centres earlier this year discovered, both have been plagued with management and organizational troubles that severely limited their research productivity in the first year.

SERC has decided to put any new centres in abeyance while it ponders what to do with its £20 million programme. "We just want to sit and think a little," says David Clark, deputy director of SERC, who runs the IRC programme. "It's an appropriate time to pause, because we're short of money." Funding shortages have forced SERC to ask the centres to cut spending by 10 per cent this year, and 5 per cent next year.

Reviewing the history of the centres has proved useful, if sobering, for SERC. Indeed, the story of at least the two IRCs under review might be a case study in exactly how not to set up an innovative new research centre. By its own admission, SERC violated several of the unwritten rules of big science manage-

ment in setting up its IRCs, and the agency is revamping the programme to avoid such mistakes in the future.

As opposed to many of the first 12 centres, future IRCs (if there are any) will:

■ Respond to an established demand.

Having a group of researchers in place and eager for a centre makes it far more likely that the centre will succeed once in place. Rumour has it that SERC set up a superconductivity IRC mainly to please then-Prime Minister Margaret Thatcher, who had seen a news article about superconductivity and called a science official to find out what Britain was doing in the area. Similarly, the idea for the engineering design centre was SERC's alone; with little established base to build on at Glasgow, it took the centre nine months just to assemble staff and find laboratory space in a nearby research park before it could do its first science. "We didn't even have room to sit, much less a place to start research in earnest," says Bernard Capaldi, who directs the 30-person Glasgow centre.

"When we set up the first centres, we did it from the 'top down', with the initiative coming from us," adds Clark. "In the future we'll do it 'bottom up', based on proposals that the community brings forward."

■ Start with strong leadership. The first two centres to be critically reviewed

The perils of industrial participation

Cambridge

As mantras go, 'industrial participation' has little competition in the world of government and academic research centres. Without a healthy dose of industry support and collaboration, current thinking goes, basic research will not become technology and technology will not become products. And that, in a time when society is increasingly asking science to prove its worth, is unthinkable. But as one UK research centre found out, industrial participation can sometimes be more trouble than it is worth.

When the UK Science and Engineering Research Council (SERC) set up an Interdisciplinary Research Centre for superconductivity at the University of Cambridge in 1988, the centre was encouraged to find at least 50 per cent of its support from industry by the end of its first six years. To help it along, SERC found it a director who not only came from industry, but would also bring some expensive equipment with him. Peter Duncan had been research director for Tube Investment Ltd, a UK technology company. When it went out of the research business, he and the surplus Tube Investment electron microprobe analyser

moved to Cambridge.

Things would never again look so good. Duncan soon left, and the microprobe analyser he brought with him turned out to be 15 years old and every bit as unreliable as a machine of that age might predictably be. How useful is it? "It serves us in a limited way," says current director Yao Liang, after some pause.

Liang, a well-respected Cambridge solid-state physicist, replaced Duncan in 1989. But the equipment problems were just beginning.

The UK company GEC offered the centre what appeared to be a real prize — a molecular beam epitaxy (MBE) machine, worth more than £300,000, that the company was no longer using.

MBEs can create films one atomic layer at a time, and are invaluable for creating new superconducting circuits. But there was one small catch: the machine had been contaminated with cadmium and mercury and would require cleaning. It took a postdoctoral researcher 18 months of scrubbing with steel wool before the MBE was free of the toxic chemicals. Total cost to the centre: hard to estimate, but at industrial rates at least £100,000.

Even new equipment found ways to complicate the infancy of the centre. When Cambridge decided to buy a £100,000 SQUID (superconducting quantum interference device) magnetometer to measure tiny magnetic fields, it had a choice of proven machines from US companies, or a half-working prototype from a Cambridge company called Cryogenic Consultants. Officials at the centre decided that it would be politic to buy British, so Cambridge selected the UK company, to its subsequent regret. Cryogenic Consultants delivered the magnetometer two years late and laboratory staff are still trying to get the machine to work properly. "It was competitive on paper," is the best Liang can say about it.

At its two-year mark, the Cambridge centre claims about a 30 per cent industrial share in its £6 million a year support. But about half of that, says Liang, is "in kind" — equipment, goods and non-monetary contributions; the stuff of headaches, if experience so far is any guide. Perhaps the best rule for future industrial participation might be one of the oldest: cash only, please.

C.A.

also happen to be the two that were announced before their directors were appointed. With such a weak start, it is not surprising that management at the two centres has been troubled ever since. When the Cambridge centre finally found a director — Peter Duncan, who came from the UK technology company Tube Investments Ltd — he soon turned out to be unsuitable, not because he was not an experienced manager, but because he had little background in superconductivity.

Although he was replaced in 1989 by Cambridge superconductivity researcher Yao Liang, morale at the centre remains low. The SERC review was triggered when Peter Edwards, one of four deputy directors (three too many, say some centre researchers) decided to leave for the University of Birmingham earlier this year.

■ **Be more centralized.** The four disciplines (chemistry, physics, material sciences and earth sciences) from which the 50-person Cambridge centre gets its researchers are located in four different parts of the university campus.

That means "a lot of pedalling" for the bicycling few who have the endurance and enthusiasm to collaborate outside of their own discipline, according to one scientist.

Next month, much of the centre will be moved into a new £1.5 million building, but many individual researchers will remain in their far-flung departments, making their way to the central laboratory as best they can.

SERC will decide what to do with the Cambridge and Glasgow centres in the autumn, after its funding council examines the reports from the review groups. Several of the other centres are also in line for review.

Like many other large science efforts, the IRC programme is controversial and has drawn the wrath of researchers who claim that the IRCs take money away from individual investigators. SERC, however, maintains that this is not the case, and while SERC insists that it found the money elsewhere. In a time of tight UK research funding, there are many who would like to see it cut to release funds for new grants. The Cambridge centre, for example, consumes one-third of the entire UK superconductivity budget.

Sir Mark Richmond, the chairman of SERC, is known to be less enthusiastic about the programme than was his predecessor, Sir William Mitchell, who started it. And industrial participation, at a programme-wide average of about 30 per cent, has not been up to expectation, in part due to the UK recession. Short of an economic turnaround at SERC, the current IRCs may be the last.

Christopher Anderson

Ozone hits new low

STRATOSPHERIC ozone over Europe has decreased by 8 per cent in the past decade, a decline that goes well beyond predictions, according to a new UK Department of the Environment study. The new figures — based on satellite measurements — show that the rate of depletion has doubled since 1980, compared to the previous decade. John Pike, head of the UK Stratospheric Ozone Group, which published the report, suggested that ozone levels could drop by 15 per cent of 1980 figures by 2000 in the latitudes between Spain and London. In April, a US study found ozone over the United States was also disappearing faster than expected, although the decline — 5 per cent since 1978 — was measured in winter and cannot be directly compared with the UK figures, taken in spring. C.A.

ERS-1 off at last

FLYING without insurance and with only a 4-minute launch window, Europe's first Earth Remote Sensing (ERS) satellite beat the odds last week with a nearly flawless launch following a 2-month delay. Systems testing and instrument calibration should occupy it for most of the week, but ERS-1 will then begin to receive data from its principal instrument, a microwave device that can operate as a synthetic aperture radar. Other on-board instruments will conduct environmental surveys using radar altimetry and devices to measure sea level and temperature, wind speed and direction. The scientific priority for the satellite is understanding the physical processes underlying climate change. Four microsats that were also aboard the Ariane-4 rocket will provide communication links and study bird migrations. C.A.

Waves rediscovered

NEARLY three decades of UK government programmes aimed at harnessing ocean waves to create electric power have finally produced their first working installation — a small concrete bunker in which incoming breakers push trapped air past a turbine to send some 75 kW of electricity into the local power grid.

Located on the remote Scottish island of Islay, the station is the third fully operational wave-energy power plant in the world, joining one in Norway and another in Japan. Between 1974 and 1983, the UK Department of Energy spent more than £15 million researching several wave power designs that were eventually discarded after a heavily criticized review found them uneconomic. Funding for wave power then fell by about a factor of 10. Now the department is redoing that study, taking into consideration future restrictions on greenhouse gases, realistic fuel costs, and new technology. C.A.

In-depth probe

SEEKING to end the 'stigma' of research on Scotland's Loch Ness, UK biologists last week announced a new project to explore the lake, which, as well as being the legendary home of 'Nessie', the Loch Ness monster, is the UK's largest body of fresh water. The only previous study of the lake's topology was done in the early years of the century and even its depth is known only to be 'at least' 750 feet.

"It is time that Loch Ness was fully explored, not by publicity seekers but by scientists," said Nicholas Witchell, who co-founded Project Urquhart, the £2 million, 3-4-year effort, which is sponsored in part by the Natural History Museum. This is not to say that the scientists would ignore Nessie should it turn up. "There are several interesting observations that have yet to be explained," Witchell said. In fact, said Colin Curds, a museum zoologist, "it is highly likely that species new to science will be discovered during our studies." He has diatoms and worms in mind, but who knows? C.A.

Limits to *Endurance*

BRITAIN'S oldest Antarctic support ship, HMS *Endurance*, is rotting and rusty and may be condemned after a dry-dock inspection is completed, and battle lines are already forming over a replacement. Should *Endurance* be decommissioned, the British Antarctic Survey wants a replacement — preferably something similar to the *Polar Circle*, a ship built by a Norwegian company several years ago for US consideration. The US National Science Foundation leased a US-built boat instead, and the *Polar Circle* could be obtained and converted for UK research purposes for about £23 million. Military officials, however, want to apply *Endurance*'s operating costs to offset budget cuts, and use military guard ships already assigned to the Falkland Islands for Antarctic duty. The military position is improved by the fact that a new airstrip on the Antarctic Peninsula could soon take over the simple transportation duties of *Endurance*. C.A.

Parallel boost

UK computer research received a boost this month when two government funding agencies announced the creation of new parallel computing centre at four universities, part of a £34-million, four-year programme that takes advantage of industrial interest — and willingness to invest — in the growing field of multiple-processor machines. Joint government and industry funding (with about £21 million from industry) will increase staff and buy computer hardware for centres at Oxford University, the University of Edinburgh, and consortia in London and Southampton. C.A.

At odds over big science

Tokyo

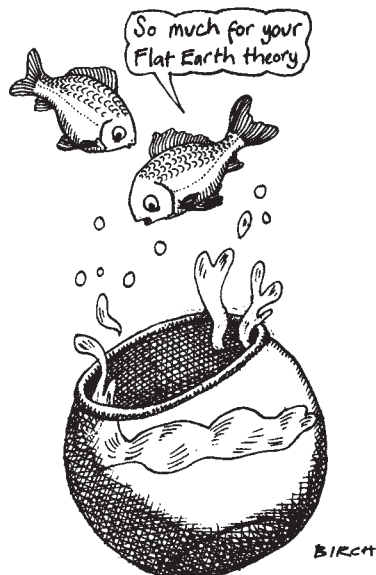
US AND Japanese government officials met in Tokyo last week for a marathon five-day session — to discuss collaboration between the two countries. The main conclusion to be drawn is that as long as the two countries stay with small joint projects, they work well together. But attempts to launch 'big science' projects are still having considerable difficulty getting off the ground. The five-day working-level meeting, held under the auspices of the 1988 US-Japan Science and Technology Agreement, drew a US delegation of twenty, led by John P. Boright deputy assistant secretary of state for oceans and international affairs.

The two countries agreed to start 33 new joint projects in the six fields covered JAPAN

Microgravity on tap

Tokyo

OFFICIALS of the Ministry of International Trade and Industry (MITI) gathered in Japan's northern island of Hokkaido last week to watch a 5-ton capsule being dropped nearly a kilometre down an old coalmine shaft. A bowl of goldfish in the rocket-shaped capsule experienced a momentary spell of near-zero gravity. The experiment, which caused some of the goldfish to swim upside down, marked the opening of the world's most advanced microgravity centre.



The Japan Microgravity Center in Kamisunagawa was built for several tens of millions of dollars with the support of the local government, private companies and MITI's New Energy and Industrial Technology Development Organization. It will be rented out for microgravity experiments to develop new materials and for the high-purity separation of cells and proteins.

David Swinbanks

by the agreement — information sciences, manufacturing and process control, geosciences, joint database development, advanced materials and life sciences. Among the most significant agreements was a decision to set up a joint US-Japan panel on volcano disaster prevention, an agreement spurred in part by the recent eruptions at Mount Unzen in Japan and Mount Pinatubo in the Philippines. The life-sciences liaison group also came to an agreement on how to divide roles between the United States and Japan in the human genome project.

There was no substantial agreement, however, on proposed collaborations in big science. The US delegation repeated US hopes that Japan will make a large financial contribution to construction of the US Superconducting Super Collider, and the Japanese responded by repeating views expressed a week earlier by Prime Minister Toshiki Kaifu to President George Bush (see *Nature* 352, 177; 18 July 1991). They said that Japan is struggling to rehabilitate its national universities and cannot say anything about a financial contribution to the project at this stage. This is about as close as Japanese diplomacy ever comes to saying no.

Japan's proposed Intelligent Manufacturing System project, a project to develop the automated factories of the future, remains up in the air waiting for the US Department of Commerce to propose terms of reference for an international feasibility study. Similarly, Japan is waiting for the same US department to propose how (and whether) with collaboration in Japan's planned sixth-generation computer project.

US officials told Japan that the United States cannot make any substantial financial contribution to Japan's Human Frontier Science Programme until at least 1993.

Japan expressed concern about the uncertainty surrounding the US Space Station project, in which Japan is committed to invest hundreds of millions of dollars to build a Japanese module for the station. US officials said there is still strong support in the US Congress and Administration for the project and Japan should not worry about a recent vote by a committee of the House of Representatives to terminate the budget for the station. The vote, although later overruled by the full House, shook Japan and may have killed any hope of a Japanese contribution to the Superconducting Super Collider.

In the light of these problems, both sides agreed that consultation in the early stages of proposed projects is essential for successful collaboration in big science.

David Swinbanks

AIDS in Yanomami?

Sao Paulo

THE Brazilian press reported last week that a Yanomami woman has died of AIDS and that others of the Indians showed antibodies against human immunodeficiency virus. The Yanomami are the largest tribal group in the Americas that is still relatively free of contact with settlers. There are about 20,000 of them, roughly divided between Brazil and Venezuela.

Although the Brazilian government has denied the report, insisting that the woman belonged to another tribe and that the reports of antibodies were caused by misunderstandings, it is undeniable that the Yanomami could have been exposed to AIDS via the 10,000 gold miners who have come into their lands and brought malaria and venereal disease.

Between January and June, 62 Indians died from these diseases in the state of Roraima, near the Venezuelan border, where many of the miners are.

Last week, Brazilian federal police and FUNAI, the Indian protection agency, began to expel the miners from the lands of the Yanomami, in the state of Roraima. But it will be too late for some of the Yanomami.

Ricardo Bonalume

INDIA

Atomic lab flooded

Bombay

THE Bhabha Atomic Research Centre (BARC) in Bombay is struggling to recover from damage caused by muddy rain water that entered the basement of a half-kilometre-long laboratory block, ruining valuable equipment. The Modular Laboratory is BARC's main building, with offices on the upper floors and laboratory facilities in the basement. On 8 June, after unprecedented monsoon rain and a landslide from an adjoining hill, the building's entire basement was under two-and-half feet of water mixed with mud.

The list of equipment damaged includes an electron linear accelerator imported from Britain, several spectrometers and a uranium laser separator used to produce 30 per cent enriched uranium.

R. Chidambaram, director of BARC, estimated the loss at Rs5 million, but unofficial sources put it at not less than Rs100 million. Chidambaram said that half of the damaged equipment had already been salvaged and that all the facilities would be brought back into operation in two months. Some senior scientists at BARC, however, dismiss this claim as unattainable. Many pieces of equipment are beyond repair because of sand inside sensitive components, and there is a general feeling that research in at least some divisions will be set back by two or three years.

K. S. Jayaraman

Biotech grows in Hong Kong

Hong Kong

SEVERAL Asian governments regard biotechnology as an obvious successor to consumer electronics in their struggle to succeed in the world's high-technology markets. The pursuit has plenty to recommend it to nations that are relatively poor and often short of land. After all, biotechnology is scientist-intensive — research and development accounts for about 80 per cent of overall costs — and it does not require huge amounts of capital or working space.

For several years, Japan has played a small but significant role in the global biotechnology business. More recently, the governments of Singapore and Taiwan have taken measures to encourage the growth of the biotechnology industries. Singapore has set up an Institute of Molecular Biology to undertake basic research and biotechnology, while Taiwan has funnelled several million US dollars into biotechnology-related industries such as agriculture and pharmaceuticals.

But the first effective biotechnological products to emerge from Asia that are not Japanese may well come from Hong Kong, the *laissez-faire* British territory that is due to revert to China in 1997.

Three years ago, a small group of scientists founded the Hong Kong Institute of Biotechnology (HKIB). A year later, the non-profit institute received \$22 million in funding from the Royal Hong Kong Jockey Club. This year, HKIB has started to produce results — a joint venture with the US pharmaceutical company Syntex that will search for new pharmaceutical products, and a memorandum of understanding with Radian, another US company, for a venture that will offer environmental technology services to local companies.

The leaders of HKIB share a strong international outlook. Dominic Lam, the 43-year-old institute director, has an array of academic titles in the Houston area, including director of the Baylor College of Medicine's Center for Biotechnology and adjunct professor of neurobiology and anatomy at the University of Texas Health Science Center, and he owns or partly owns five biotechnology-based companies around Houston.

HKIB's associate director, Siu-Leung Lee, has a similarly broad background. With a bachelor's degree in biology from the Chinese University of Hong Kong and a PhD in biochemistry from Purdue University in the United States, Lee has held academic posts at Yale and Texas A&M Universities. In 1982, he went into industry, leading a research group at Corning Glass Works, and two years later moved to Battelle Memorial Institute to set up a bioseparations laboratory. He returned to a full-time job at HKIB in 1989.

A major reason for the founding of the HKIB was the prospect that it would serve as a gateway to mainland China, which has both vast resources of natural products and a large reservoir of excellent biotechnologists. Indeed, Lam characterizes China as the sleeping giant of the Asian biotechnology business. "China offers more of an opportunity in developing biotechnological products than anyone else in the world, including the US," he said.

Even the current political climate has not dimmed that prospect, he said. "To a large extent, the good people in biotechnology move up, and they have a fairly decent peer review system."

HKIB's joint venture with Syntex, called HKIB/Syntex, will use Chinese resources in its first project. The objective is to screen synthetic and natural products from China — particularly those used in traditional Chinese medicine — for pharmaceutical potential. Scientists at HKIB and Syntex Research in Palo Alto, California, will carry out the screening. The raw materials will be gathered by researchers associated with the Chinese Academy of Sciences.

HKIB is also acting as the middleman in a more local project, designed to clear indigo dye from Hong Kong's waterways. The territory's garment industry uses about 4,000 tons of dye annually, to make blue jeans blue. According to Chinese University microbiologist Kai Keung Mark, about one-tenth of that ends up in the waterways, which it stains an unpleasant blue/black colour.

Two years ago, Mark discovered a natural strain of bacteria that degrades indigo, converting it into colourless, non-toxic compounds. Now, Mark and HKIB are undertaking research on the compound. Investigations include determining how much the bacteria produce of the enzyme responsible for decolorizing indigo and analysing how long the bugs can withstand high concentrations of indigo in waste water from garment factories. Biologists hope that genetically engineered versions of the bacteria could replace the process of stone washing to give jeans the fashionably faded look, and could even remove the indigo from waste waters before they reach waterways.

HKIB's other main initiative, in cooperation with the US company Radian, shows the institution's pragmatic side. The aim is to provide clean-up services, such as water treatment, for Hong Kong companies, and the joint venture will not restrict itself to biotechnology-based services. It is prepared to offer whatever technology is needed for the jobs at hand. "We're problem oriented, not technology-oriented", explained Lee.

Peter Gwynne

Science park in Macao

Hong Kong

THE government of Macao, the Portuguese colony across the Pearl River estuary from Hong Kong, plans to establish a new science and technology park with help from a newly formed Sino-Singaporean joint venture. The park, which will cost several hundred thousand US dollars, will be built on 3.4 million square feet of reclaimed land on the Macao island of Taipa.

Macao normally lives in the shadow of Hong Kong, but this year the Portuguese enclave has made technological headlines of its own. In March, the United Nations University opened its International Institute for Software Technology in Macao. The object of the institute is to help research, development and advanced training needs of developing countries. The new science park is a further indication of the colony's commitment to technology.

Details of the park have not yet been released. However, Long Zhu Science and Technology Company, the Sino-Singaporean joint venture helping to finance the park, has said it will use China's technological capabilities to foster the growth of the park. Macao is due to revert to China in 1999, two years after Hong Kong.

P.G.

Hong Kong university

Hong Kong

A NEW science-directed university will open its doors in Hong Kong in October. The Hong Kong University of Science and Technology will join two other universities and two polytechnics that offer scientific training in this British territory of six million people.

Establishment of the university, which was officially approved in 1988 after several years of debate, is a response to concern that Hong Kong is falling behind other Asian nations in its development of technology. Close to 80 per cent of the territory's electronic companies have fewer than 50 employees, and so cannot afford to carry out research on new products. Meanwhile, countries such as Malaysia and Thailand are undercutting Hong Kong's traditional advantage of cheap labour for assembly of high-technology products. Hong Kong's government has for many years taken a *laissez-faire* attitude to industrial policy. But recently it has recognized the need to support industry indirectly, by providing high-quality higher education in science and technology.

The new university expects to open its doors to 700 students, including 560 graduate students. It will have more than a hundred faculty members in departments of biochemistry, biology, chemistry, computer science, electrical and electronic engineering, mathematics, mechanical engineering and physics.

P.G.

Asian elephant threatened

SIR — Pagel and Mace¹ present a strong argument in favour of the current ban on trade in elephant products. The halving of the African elephant population in the past eight years is justly a cause for concern. But we wish to draw attention to a generally overlooked aspect of elephant conservation — that there are two species of elephants, and that the CITES ban has minimal benefits in the conservation of the Asian species, *Elephas maximus* L.

The Asian elephant is much rarer than its African cousin. Current estimates are imprecise, but put the number of wild Asian elephants at 34,000–56,000 with a further 16,000 in captivity². Even optimistic figures indicate that there are only one tenth as many Asian as African elephants^{2,3}. Asian elephant numbers may not have undergone a dramatic decline in recent years, but the species faces much more intractable conservation problems.

Elephant poaching may be a relatively minor problem in Asia today and, because some males and all females lack tusks⁵, poaching cannot be the terminal threat it is in Africa. Much more important for the Asian elephant are habitat loss and fragmentation as a result of escalating human population, which in turn leads to increasing conflict between man and elephant. In India, for example, which may contain half of all wild Asian elephants, the human population increased from 236 to 790 million in the period 1901–88⁴. This increase places intense pressure on undeveloped areas. Only one-third of Asian elephant habitat is in protected areas⁴.

Erosion of habitat forces elephants into agricultural areas, where they destroy crops and inevitably cause human fatalities: 150–200 a year in India⁴. Most remaining populations are already small. Fragmentation of habitat leads to fragmentation of elephant populations. In Thailand, 29 protected areas hold 1,300–1,700 elephants, but only 13 of these areas hold more than 25 individuals⁴. Many Asian elephant populations in the longer term may not be viable^{2,6}. It does not help that many of the larger elephant populations are located in politically unstable areas — for example the 8,500–11,000 elephants in northeast India². The destruction wrought in Manas National Park, Assam, by Bodo secessionists shows how vulnerable even protected areas are to political unrest. In the Vietnam war, US forces bombed elephants because the Viet-Cong were using them as transport⁴.

None of the problems faced by the Asian elephant can be alleviated overnight as poaching has been in Africa. Neither has much effort been expended

by the international community in finding ways in which human and elephant populations can successfully co-exist (although the individual countries involved are trying to tackle these problems). This state of affairs must be rectified, because the continued success of the CITES ban, coupled with human population growth, will result in the Asian situation being repeated in Africa in the near future. It will be in the long-term interests of both elephant species to find solutions to the Asian elephant's problems now.

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Origin of bacterial adaptation

SIR — Harry Rubin's letter¹ is welcome in pointing to the stimulating contribution of the late Sir Cyril Hinshelwood to debates in the 1940s and 1950s on the origin of bacterial adaptation. Rubin does not, however, make clear why this contribution was less helpful than it might have been.

As Rubin correctly says, Hinshelwood did not argue that adaptation in bacteria could never arise by mutation, and indeed sometimes accepted this as an explanation². He was, however, more concerned with phenomena, in particular the 'training' of bacterial cultures to increasingly higher levels of antibacterial agents, where he considered this explanation unlikely. His reasons for questioning it in such contexts remain cogent, and it is true that no complete explanation of the observations is available even now, although it could be argued that he remained unwilling to accept fully the implications of the likelihood of polygenic drug resistance even when evidence in accord with this was obtained in his own laboratory³.

However, the features alluded to by Rubin, namely "the speed of change, the large fraction of the population involved, the finely graded nature of the response,

the lack of precise specificity and the reversibility of the altered state", do not have to possess an origin in self-adjusting changes in flow through different reaction networks, as Hinshelwood supposed. One is inclined nowadays to suspect the involvement of stress responses mediated by specific control mechanisms, such as the heat-shock system.

We are perhaps used to thinking of such systems, especially in bacteria, as solely providing quick but rapidly reversible responses. But there is no reason why other sorts of kinetic behaviour should not occur, as pointed out long ago by Monod and Jacob⁴ (see also the reference to an earlier model by Delbrück on page 398 of this citation). It may be that Hinshelwood's recognition of this led him never to dispute the *lac* paradigm directly (as far as I know). (That we usually do not consider the potentially flexible kinetic behaviour of even moderately complex control systems mediated by specific molecular mechanisms is probably due to the disinclination of those working in this area, such as myself, to do much mathematical modelling.) There seems no reason in principle why such responses might not be involved in the extremely interesting phenomena described by Rubin⁵ as well as in the other cases he refers to in his letter.

In fairness to Hinshelwood, it should be pointed out that he was remarkably tolerant of work in his own laboratory, such as my own modest effort⁶, that seemed to go against his own views.

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Grant to HUGO

SIR — In two recent articles on the Human Genome Project (*Nature* **352**, 3 & 11; 1991) you stated that the Soviet government was the only government or public grant-making agency to have funded HUGO (the Human Genome Organisation). This is not true and I would be grateful if you would set the record straight. The Australian government has already made a grant of \$A50,000 to HUGO.

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Shake-up for earthquake prediction

Robert J. Geller

Japan's ambitious earthquake prediction programme has no hope of success, unless fundamental research is emphasized and data are shared.

"At present there is no possibility of earthquake prediction . . . Attempts at [earthquake] prediction have had [little significance] in relation to the actual development of our knowledge and understanding of earthquakes" (C. F. Richter, 1958, p.385-387¹).

THIRTY years ago, in a bold departure from the then conventional wisdom¹, Japanese seismologists proposed a large-scale earthquake prediction research programme². Similar programmes in the United States, Soviet Union, China and elsewhere were established within the next few years. Unfortunately, neither the Japanese programme nor similar projects in other countries have achieved significant progress. Even now, no consensus exists on whether earthquake prediction is possible, even in principle.

I believe that the following drastic changes should be made in Japan's earthquake prediction programme: (1) the public and government should be clearly informed that earthquake prediction is presently not possible; (2) earthquake prediction and related basic research should be tightly linked, rather than treated as separate entities; (3) a full and open review of earthquake prediction research should be conducted; and (4) all data gathered by the earthquake prediction research programme should be made freely available.

Is earthquake prediction possible? As defined by Wood and Gutenberg³ in 1935, an 'earthquake prediction', as opposed to a mere 'generalized forecast', must specify the region, the time and the magnitude of the event; the first two must be given within narrow limits. Allen⁴ specified three additional criteria that a statement regarding future seismic activity must meet to qualify as an earthquake prediction: it must indicate the author's confidence in the prediction; it must indicate the probability the earthquake would occur anyway, as a random event; and it must be written and presented in a publicly accessible form.

Blueprint

The bulk of the original 1962 "blueprint" for earthquake prediction research in Japan² presented a reasonable programme for augmenting observations of crustal deformation (through geodetic, tide gauge and continuous observations), microearthquakes, changes in intrinsic seismic wave velocities (through control-

led experiments using explosive sources), active faulting, and geomagnetism and Earth currents. Whether these data could actually be used to predict earthquakes was discussed only briefly: "... It seems highly probable that we would be able to find some significant correlation between earthquake occurrence and observed phenomena merely by accumulating data for several years". The US earthquake prediction research programme was established on the basis of a similar empirical approach⁵.

The empirical approach depends on the existence of reliably measurable and unambiguously identifiable precursors. There might arguably have been reasons for supposing, in 1962, that such precursors existed, but there is no longer room for such a belief.

Many possible precursors have been considered by researchers throughout the world, including temporal variation of seismic velocities, crustal uplift, tilt and strain data, temporal variations in the pattern of seismic activity, electrical and magnetic anomalies, water well level changes, temporal variation of the isotope composition of ground water, and even animal behaviour. Each of these possible anomalies has been the subject of encouraging reports at one time or another, but subsequent studies have raised doubts. Suzuki's review⁶ of earthquake prediction (both inside and outside Japan) still seems applicable: "... The present state of the art is very chaotic... A remarkable variety of earthquake precursors have been reported so far. Some reported precursors seem very strange and open to doubt. Even excluding these ambiguous cases, no general and definite way to successful earthquake prediction is clear. No panacea is suggested even from the experiences in successful predictions." A recent analysis of a database of more than 1,100 published reports of precursors⁷ is consistent with Suzuki's pessimistic view.

The difficulty of making empirical earthquake predictions can also be seen by considering earthquake 'post-prediction', that is, the retrospective examination of data to identify precursory anomalies that could in principle have been identified before the event. Earthquake post-prediction is much easier than earthquake prediction, because there is no 'false alarm' problem. The examples presented in both Suzuki's review and a more recent review by Mogi⁸

suggest that even routine empirical post-prediction is not presently possible; thus there is no reason to believe that empirical earthquake prediction is possible.

In both Japan and California we have an excellent understanding of the overall tectonic setting (plate boundaries and rates). This enables seismologists to make generalized forecasts of future seismicity. However, in both the United States and Japan, there recently has been a tendency to call such forecasts "long-term earthquake predictions." This tends to obscure the fact that earthquake prediction, as originally defined by Wood and Gutenberg³, has proven to be extremely difficult. For example, the Parkfield, California Earthquake Prediction experiment⁹ should probably be classified as a generalized forecast rather than as a prediction.

Empirical earthquake prediction seems to have come to a dead end. If there were obvious and reliable precursors they probably would have been found a long time ago. Developing a better understanding of the physics of the earthquake process is essential in order to determine whether or not earthquakes can be predicted. More emphasis should be placed on basic research in seismology, geodesy, tectonics and other related fields; the empirical approach should be de-emphasized.

In 1978, the Large-scale Earthquake Countermeasures act was enacted by the Japanese parliament. In accordance with this law, the Japan Meteorological Agency (JMA) and other government agencies continuously collect, record and monitor seismological data. Particular emphasis is placed on the Tokai district (along the Pacific coast, south of Tokyo) in which, based on historical seismicity, a great earthquake in the relatively near future is considered probable.

If apparently anomalous data are recorded, an Earthquake Assessment Committee consisting of six university professors will be convened at short notice to review and assess the data. If the committee decides that the anomalous data should be interpreted as an earthquake precursor, they will advise the cabinet to issue a public warning. The committee has never been convened operationally, but routine meetings are regularly held.

The national government of Japan, and prefectural and local governments in and near the Tokai area, have prepared

a thorough system for disseminating the cabinet's warning of an impending earthquake. Tokyo is not included in the Tokai district, but similar precautions have been taken there. These precautions extend to the setting up of response plans within individual companies, schools and other organizations. For example, there is a poster at Tokyo University, prominently displayed in many locations, instructing employees and students on the procedures to be followed in the event of an earthquake warning.

The public knows that a system for disseminating warnings of an impending earthquake in Tokai has been established, and hears optimistic statements by representatives of the earthquake prediction research community, such as "Once it is known where earthquakes are likely to occur, the next question is when they will strike. If the location has been pinpointed accurately, various kinds of networks can be established to observe the precursory phenomena that make it possible to predict an earthquake four to five hours before it happens"¹⁰. Such statements tend to foster the misleading impression that we are able routinely to predict earthquakes.

After the Niigata earthquake in June 1964, the Japanese government approved a four-year plan for basic research on earthquake prediction, based on the 1962 "blueprint". The first four-year plan began in April 1965. Subsequent plans have been five-year plans; the present, sixth, five-year plan runs until March 1994. The first four-year plan was called a plan for earthquake prediction research, but the word "research" was dropped after this.

Several government agencies participate in the earthquake prediction programme. These include not only national universities, and research institutes affiliated with the Ministry of Education, Science and Culture (*Monbusho*), but also the JMA, the Maritime Safety Agency, the Geographical Survey Institute, the Geological Survey of Japan, the National Research Institute for the Earth Sciences and Disaster Prevention of the Science and Technology Agency, and the Communications Research Laboratory of the Post and Telecommunications ministry. In this article I focus on national universities and other *Monbusho*-affiliated institutions, but these institutions account for only a fraction of the total budget and personnel involved in earthquake prediction.

The budget for earthquake prediction at *Monbusho*-affiliated institutions is currently ¥1,908 million (\$14 million) per year. An additional ¥397 million per year is appropriated for prediction of volcanic eruptions; this programme is in practice highly integrated with the earthquake prediction programme. Most of

the budget is allocated to routine observations, for example, leasing telephone lines for telemetry. As one might expect, vested interests have become strongly entrenched. As the budget is not growing faster than inflation, new activities cannot be funded without curtailing existing ones; the programme has thus become essentially frozen.

The Geodetic Council of Japan is an advisory committee — "*shingikai*" — which makes recommendations to *Monbusho* concerning all fields of Earth sciences, not just geodesy. Its subcommittee for earthquake prediction, which consists mainly of people closely associated with the existing earthquake prediction programme, reviews the programme every five years. But reviews tend to be superficial and do not examine the fundamental premises of the programme.

Data collected under the programme are in many cases not open to outside researchers. Even when they are, there may be a long delay, and the data may not be available in the most convenient form. An example is the catalogue of hypocentres and travel times published by the Earthquake Prediction Data Centre of the Earthquake Research Institute of Tokyo University. This catalogue is compiled from data provided by regional data centres and observatories of various national universities. At the insistence of some of these regional institutions there is a substantial delay in publication (the bulletin for the second half of 1987 was published only in March 1991), and the bulletin is distributed only in hard-copy format. Outside researchers who want to analyse data from the bulletin (which was of course produced by a computer) have had to key in the data from hard copy. The situation will improve later this year when the bulletin should become available in a machine-readable format. But the limited availability of data is still in great contrast to the field of global seismology, where data are open to all seismologists in accordance with procedures established by the Federation of Digital Broadband Seismograph Networks (FDSN).

I would like to recommend the following changes to Japan's earthquake prediction programme. (1) The government and public should be clearly informed that earthquake prediction is not possible, and that the goal of earthquake prediction research is to advance the state of the art of seismology and related

fields to determine whether (and, if so, how) earthquake prediction is possible. A careful reading of statements issued by the earthquake prediction programme would show that the Tokai earthquake is the focus of the Earthquake Assessment Committee's activities, and that even there the prediction of an earthquake has not been guaranteed. However, the public, government and media may not be aware that this is the case; the present inability to predict earthquakes should be clearly communicated before the next large damaging earthquake.

(2) Earthquake prediction and basic research in seismology and other related fields should be closely linked. It is important to study earthquakes and Earth structure globally, not just within Japan, if the state of the art in seismology is to be advanced.

(3) The seventh five-year earthquake prediction plan (which will begin in April 1994) should not be a mere continuation of the sixth five-year plan. A full review, open to all specialists in seismology and related fields, should be conducted; selected researchers from outside Japan and from other fields should be invited to participate. The scientific objectives of earthquake prediction research should be redefined. The elements of the seventh five-year plan should be based on the scientific objectives; existing activities should not be automatically continued.

(4) All data should be made freely available to all researchers. Similarly, new theoretical results and computer codes for data analysis should also be made freely available to all researchers. Following the example of, say, the Deep Sea Drilling Project, it might be reasonable to allow people who collected the data to have priority for six months (or even one year), as long as all data are unconditionally open after this period.

The empirical approach to earthquake prediction has not been fruitful, but I believe that fundamental reform of the earthquake prediction programme could lead to exciting new research that would yield a better understanding of the physics of the earthquake source process. Such a programme would make important scientific contributions, and also would contribute to society through the mitigation of earthquake hazards. □

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Non-locality bursts into life

The past few years have seen a resurgence of interest in the foundations of quantum mechanics, not least because there has been a succession of generations.

THAT we seem to be in for another bout of argument about the interpretation of quantum mechanics is plain, to judge from what has been appearing in the physics journals, but why now, more than half a century after Nils Bohr is believed to have settled everything? There are several explanations. Bohr did not quieten all doubters, among whom Einstein was one (most often represented by the *gedanken* experiment due to Einstein, Podolski and Rosen, or EPR for short). Nor was de Broglie ever reconciled to the Copenhagen view; there remains an active school of researchers devoted to his view.

Much more recently, it has been recognized that Bohr plucked from thin air the assumption that the square of the (generally complex) amplitude of a solution to the wave equation represents the probability distribution of the particle or other entity concerned, however successful interpretations based on it may be. The recent resurgence of interest thus at least partly springs from the drive to make such leaps in the dark intelligible.

This seems the spirit in which people have been testing the old principle that quantities or "observables" represented by mathematical operators that do not commute with each other cannot be measured simultaneously. From that it follows that it is possible to know only two components of the angular momentum of a particle, perhaps the components in two distinct directions, but more usually the component in one direction and the total angular momentum (which is a number).

The now-standard drill for doing that stems from the fact that any relationship between operators representing physical observables must be mirrored in an identical relationship between values assigned (as if by measurement) to the same quantities, when it becomes a matter (not always simple) of showing that the allowable numbers cannot be consistently assigned to all of a set of physical variables.

But a more immediate stimulus of interest among experimentalists in the interpretation of quantum mechanics has been the publication (itself now more than a quarter of a century ago) of the late J. S. Bell's now-famous inequality — a numerical relation between the probability that physically distant events will be correlated with each other.

Non-locality is the new game, as the EPR experiment dictates. Einstein was not the only one offended that the several

components of a single well-defined quantum state may be physically separated yet still bound together by the overall properties of the quantum state.

A simple example is that of two electrons more or less at rest in a singlet state, which means that their net spin momentum is zero, or that (if the term has meaning) the two electron spins are oppositely directed. So what happens if the two electrons are flung diametrically apart by some agency or another? Although the random direction in which the two spins cancel is still undetermined, the cancellation persists indefinitely. And if, at some stage, the projection of the angular momentum of one operator in some direction is measured, one can confidently infer the exact opposite for the corresponding component of the other.

Einstein's protest was that this state of affairs cannot make sense. By what physical means can one electron "know" how to satisfy the overall requirements of the quantum state once a decision has been made to measure the angular momentum of its partner? Whence (in part) the doctrine of hidden variables — the assertion that each of the two separating electrons must carry the information determining its behaviour, consistently with that of its partner, when it encounters a piece of equipment that requires the direction of its spin to be declared.

Attempts to investigate this behaviour experimentally are bound, of course, to be frustrating. With the same singlet state of the electron pair, one can think (most of this is still *gedanken* stuff) of using a strong magnetic field to allow electrons to pass if they are polarized in the direction of the field, but which rejects them if they are oppositely polarized. One can be sure that both members of a singlet electron pair will each pass through a magnet in its path only if the fields are in opposite direction.

Most simply, the outcome of such an experiment can be represented by the product of two numbers, one for each magnet, in which one scores +1 for passage and -1 for failure. Allowing for arbitrary directions of the magnetic field, say **a** and **b**, the general quantum result is that the expectation value of the product of these two numbers (or the average over many individual measurements) is simply the *negative* of the scalar product **a.b**.

Bell's tongue-in-cheek inequalities arise from supposing that the two electrons leave the tightly bound singlet state

with a nearly perfect memory of where they have come from, but that they then evolve independently. The numerical inequality is derived by a comparison of the two states, represented by the directions **a** and **b**, with a third, say **c**. The correlation (or anticorrelation) is less strong.

Daniel I. Fivel from the University of Maryland at College Park has now brought many of these arguments together in what seems to be a splendid piece of rationalization by generalization (*Phys. Rev. Lett.* **67**, 286; 1991). All these discussions seem to hang on correlating what happens at one measuring device with what happens at another. So the expectation value of a result with orientation **a** at one magnet and orientation **b** at the other is a number that might be thought to connect together all pairs of points on the surface of a sphere. (It turns out to be simplest to get rid of negative numbers by adding +1 to the expectation value and dividing the result by two.)

The function that results has all the properties of a metric (for measuring distances on the surface of a sphere) and Bell's inequality turns out to be just the triangle inequality (that the sum of two sides must be greater than the third). But Fivel is also able to show that the assumption that only local issues matter, perhaps helped along by hidden variables, lead to the construction of a metric incompatible with the first. It looks as if the outlook for the EPR way of working may be doomed.

But that will not prevent further experiment. Indeed the same issue of the same journal contains an argument (by J. D. Franson of Johns Hopkins University) that non-locality can be demonstrated by interference between the photons of two light beams manipulated so that they reach the detectors at slightly different times, and an intriguing experiment with a pair of nonlinear crystals (lithium iodate) by a group from the University of Rochester in which unexplained non-locality is destroyed by preventing the stimulation of one crystal by light from the other. For piquancy, there is also a protest by P.R. Holland and J.P. Vigiér from the University of Paris at what they consider to be a misrepresentation of de Broglie.

There is ample evidence that the preoccupation will not go away. This does not mean that people are worrying about the foundations, but that they wish to understand them better. A new generation has arrived.

John Maddox

It's all in the timing

David Black

THE planet-sized companion discovered by Bailes, Lyne and Shemar in orbit around the pulsar PSR1829-10 (see page 311 of this issue¹) has two claims to our special attention. First, if correctly identified, it would be the first 'planet' to be discovered outside the Solar System. And second, its parent star must have been born in a destructive supernova explosion, according to our current understanding, which the planet must have survived somehow — unless it formed subsequently or unless pulsars can evolve in some other way. Which-ever, the existence of a companion seems remarkable.

The authors infer the presence of the companion from the timing of the pulsar's radio pulses over the past five years. After allowing for the gradual slow down of the pulsar and the systematic effect of the Earth's orbit, they found an oscillatory residual behaviour with a period of about 6 months. This they attribute to motion of the pulsar as it and its small companion orbit around their mutual centre of mass.

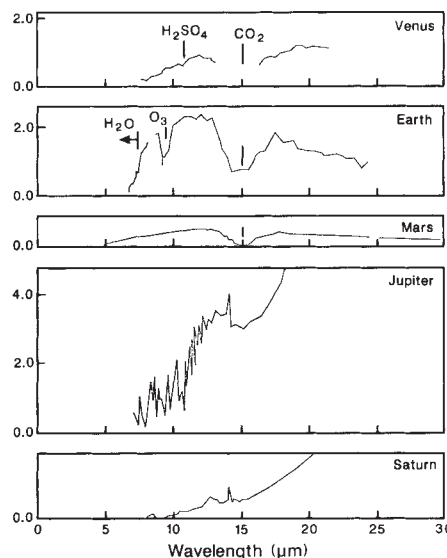
The search for planetary systems beyond our own is highlighted in the National Research Council's prospectus² for astronomical research in the 1990s as one of the key programmes. In part, this is because, over the past ten years, a consensus has emerged in the form of a paradigm for how our Solar System, and presumably other planetary systems, formed. The sequence of supposed events begins with a cold, tenuous cloud of molecular gas, a portion of which collapses gravitationally to form an accretion disk from which the central star and its retinue of planets condense. The tasks now are to formulate testable hypotheses about the properties of planetary systems in general, and so refine our view of how the Solar System evolved and relate the evolution of such systems to the birth of the dominant member of each, the central star.

It is possible to identify three phases in the search for other planetary systems. The first is the unambiguous detection of another planetary system. The new discovery excepted, that phase has not yet occurred, although there have been false starts and tantalizing hints. But as yet, there have been no confirmed detections.

The next phase, and this is when the scientific returns begin to be realized, is to determine statistical properties of planetary systems. For example, what fraction of stars of a particular spectral type has planets? Trends in such properties as the mass ratio between the star

and the largest planet will also be valuable in constraining our models.

The last phase involves gathering details about the individual planets. This would provide data regarding the mass, the orbit and the temperature of the planets. Particularly exciting would be spectral information on the planetary



Spectral signature of planets in the Solar System showing both the great diversity in planetary spectra, as well as the possibility of learning a great deal about planets revolving around other stars from relatively modest resolution spectroscopy.

atmospheres. The potential richness of such studies can be seen readily from a cursory look at spectra from planets in the Solar System (see figure). Our current understanding of atmospheric evolution is inadequate to use such spectra to constrain our view of other planets. But by analogy with our own planet, the detection of significant amounts of ozone and oxygen would be remarkable. The Earth's atmosphere is well out of chemical equilibrium because of life. The detection of similar levels of oxygen and ozone in another atmosphere would have profound implications regarding the existence of life beyond the Earth.

In observational or experimental research, important discoveries are often made by accident. The search for other planetary systems appears to be no exception. The discovery of a dusty disk structure around the AO main-sequence star Vega was made by the observers using data from the Infrared Astronomical Satellite (IRAS); Vega was being used merely for calibration purposes. The discovery using radial velocity observations by Latham and

coworkers³ of a low-mass companion to the solar-type star HD114762 was also accidental; again the object was a calibration standard. Equally, the discovery by Bailes *et al.* is a serendipitous identification during a broad survey of 40 pulsars.

From the amplitude of the oscillatory timing residuals, and on the assumption that the pulsar has the canonical mass of 1.4 solar masses, the authors estimate the companion's apparent mass to be ten times that of the Earth. The mass is 'apparent' because of the unknown inclination of the companion's orbit to our line of sight. The companion's real mass is its apparent mass divided by the sine of the angle of inclination. Bailes *et al.* argue (assuming there is no preferred orientation) that there is less than a 1 in 1,000 chance that the angle is small enough to make the real mass comparable to that of Jupiter (320 Earth masses). But it should be noted that the solar-type star studied by Latham *et al.*³ turns out⁴ to have the implausible, pole-on type of orientation towards us that Bailes *et al.* discount.

A puzzling attribute of the companion is that its orbit is surprisingly circular, given its putative history, having a relatively low eccentricity of 0.10 (somewhat less than that of Mars or Pluto, although greater than Earth's). And although the orbit, about the size of Venus's, is superficially quite small, it is still large compared with what one might expect considering the tidal effects the parent pulsar might have on the planet.

Did the companion form in the paradigm way described above, perhaps with other undiscovered planets, and survive the supernova explosion that destroyed its original parent and formed the pulsar? Or is it a manifestation of a different process, one that might resemble the standard picture, but which occurs during or after the birth of the pulsar?

If the companion is a remnant of an old planetary system that formed in conjunction with the massive progenitor to the pulsar (the progenitor mass is in the range of 6–60 solar masses), the low apparent eccentricity of its orbit places severe constraints on the nature of the pulsar's origin. The sudden loss of mass from the central star that would occur in a supernova explosion would change the companion's orbit simply through the change in gravitational pull it exerts. If more than half of a star's mass is lost in less than an orbital period, its companions would become unbound. Clearly the massive progenitor in this case lost more than half of its mass in becoming a 1.4-solar-mass pulsar. But what if the mass loss proceeded through several small, discrete stages? Although this might preserve the initially circular orbit of the companion, it would take a cor-

respondingly long time to shed all the mass lost. It seems doubtful that this adiabatic loss process could generate a pulsar from the likely progenitor. But if this "kinder, gentler" process works, the new discovery will have led to a revolution in this branch of astrophysics.

The orbit of the companion is difficult to understand even if the companion was formed around the pulsar progenitor. Such a massive, luminous object would cause planets to form at greater radii than we see in the Solar System. It is fair to say, however, that our understanding of the formation process is sufficiently poor that the nature of any scaling laws eludes us. But if the progenitor's mass was near the upper end of the postulated range, the radiation pressure from the young star could well disrupt any effort to form a planetary system.

An alternative idea noted by Bailes *et al.* is that the companion could be the remaining vestiges of a binary stellar companion of the pulsar, and that we are witnessing the demise of the last part in 10^5 of the original star. The authors' scepticism on this notion is justified.

A more likely, and equally intriguing, explanation for the companion is that it was formed during or in connection with the formation of the pulsar itself. The idea here is that a disk is formed as part of the process forming the pulsar. The companion would be formed from the disk in much the same way that it is thought the Earth formed from the solar nebula. The formation of a companion from such a disk would probably give rise to an orbit of low eccentricity. And the physical dimensions of the nebula would also be more consistent with the size of the orbit than a nebula formed with the pre-main-sequence star.

There is, of course, the possibility that there is no companion. The history of searches for other planetary systems is littered with published detections that vanish under further scrutiny. Indeed, there is a previous claim to have detected a companion⁵ (in one case three companions⁶) to the pulsar NP0532, in the Crab Nebula. It is therefore very important that this discovery be corroborated. If there is a companion, the work of Bailes and coworkers will challenge some, perhaps several, fundamental aspects of our views of the evolution of stellar and planetary systems. □

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The defect in Marfan syndrome

Victor A. McKusick

ALTHOUGH Marfan syndrome was recognized as a heritable disorder of connective tissue more than 35 years ago¹, the precise basic defect has eluded investigators. Until now that is — three papers by Lee *et al.*², Maslen *et al.*³ and Dietz *et al.*⁴, on pages 330, 334 and 337 of this issue, present the dénouement.

Marfan syndrome, estimated to affect one in 10,000 people, is named for a Paris paediatrician who in 1896 described severe skeletal abnormalities in a 5½-year-old girl. The association of dislocated lenses in the eye was noted by Boerger in 1914; the main life-threatening cardiovascular complications — aneurysm of the aorta and aortic dissection — were reported in single cases in 1943. Studies of a relatively large number of patients and their families in the 1950s delineated the natural history of the syndrome (particularly the cardiovascular complications⁵), defined the range of pleiotropism and variability, and established its autosomal dominant inheritance.

Because the disorder is caused by a single gene and abnormality is expressed in the heterozygote, a unitary defect of a structural element of connective tissue seemed likely. In 1956, it was possible to write¹: "What the suspensory ligament of the lens has in common with the media of the aorta is obscure. If [this were] known, the basic defect of [the Marfan] syndrome might be understood". The dolichostenomelia (Marfan's term for 'long, thin limbs') and the arachnodactyly ('spider fingers'), as well as the pectus excavatum and pectus carinatum ('hollow chest' and 'pigeon breast'), seemed to represent excessive longitudinal growth of tubular bones in the limbs, fingers and ribs.

In the late 1950s few would have predicted that it would take so long to find the underlying defect in the Marfan syndrome. From the example established by Vernon Ingram^{6,7} for sickle cell haemoglobin, the replacement of a single amino acid in a connective tissue protein was considered likely. This has turned out to be the case in at least some patients⁴. But because of the large size and peculiar physicochemical properties of connective-tissue proteins, methods for studying the gene rather than the gene product were required. Through molecular genetic approaches, defects in fibrous collagens have been delineated in osteogenesis imperfecta⁸, the Ehlers-Danlos syndromes⁹ and specific skeletal dysplasias¹⁰, and now in Marfan syndrome as well⁴.

The identification of the 'Marfan

gene', reported in this issue, is in part a triumph for gene mapping. In some mendelian disorders of previously unknown basic biochemical 'cause' (cystic fibrosis, for example), the first step was mapping the mutant gene by family linkage studies of the clinical disorder, followed by cloning the wild-type gene by moving in on it — a process called reverse genetics, or more appropriately, positional cloning. A second method for disease-gene identification — and the one used to tackle Marfan syndrome — is the candidate gene approach. What gene maps to the same chromosomal region as the disease phenotype? Does the gene for a protein plausibly involved in the disorder map to the same chromosomal site?

Proof of the defect in Marfan syndrome was developed as follows. First, immunohistological studies showed that fibrillin may be defective or deficient in patients suffering from the disorder¹¹. Fibrillin, a 'new' connective tissue protein, was discovered in 1986 (ref. 12). Because it is an element of elastic tissue and is abundant in tissues affected in Marfan syndrome, including the aorta, the suspensory ligament of the lens and periosteum (loosely, bone covering), the late David Hollister suspected it might be the site of the Marfan abnormality. Second, Marfan syndrome was mapped to the long arm of chromosome 15 (15q) by family linkage studies^{13,14}. Third, a probe from the partially cloned³ fibrillin gene (*FBNI*) was mapped to the same region of 15q (15q21.1) by *in situ* hybridization¹⁵. Fourth, as described by Lee *et al.*² and Dietz *et al.*⁴, a DNA polymorphism in the fibrillin gene clone was shown to be tightly linked (no recombinants identified) to the Marfan phenotype in family linkage studies. Finally, using clones of the fibrillin gene isolated by Maslen *et al.*³, Dietz *et al.*⁴ identified *de novo* point mutations in the fibrillin gene in patients with sporadic Marfan syndrome.

Marfan syndrome shows clinical variability both within and between families. Variability within families presumably results from differences in the genetic background against which the Marfan gene is operating. Variability between families may also in part have this cause, but there is a possibility in such instances that the mutations are allelic (that is, they occur at different sites in the Marfan gene), or perhaps they are in different genes. Genetic heterogeneity can be the result of different mutant alleles or mutations at different loci. Genetic heterogeneity in what

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Abraham Lincoln and Marfan syndrome

THIRTY years ago the idea that Lincoln had Marfan syndrome was raised independently by two physicians. Both based the impression in large part on Lincoln's long limbs and descriptions by his contemporaries of his loose-jointedness and unusual physique. For example the Washington correspondent of *The Times* described him as a "tall, lank, lean man, considerably over 6 feet in height, with stooping shoulders, long pendulous arms terminating in hands of extraordinary dimensions, which, however, were far exceeded in proportion by his feet". Later, other physicians and Lincoln buffs disputed the diagnosis, partly because of Lincoln's legendary strength (which we know does not exclude Marfan syndrome by any means). Nonetheless it was impossible to be certain.

With the advent of the polymerase chain reaction for amplifying trace amounts of DNA, and the identification of the nature of the gene defect responsible for Marfan syndrome (described in the accompanying article), an attempt at a definitive diagnosis can now be made by searching Lincoln's fibrillin genes for a mutation. Thinking that the defect in Marfan syndrome might lie in a form of collagen, Darwin Prockop, a leading student of connective tissues, last autumn approached the National Museum of Health and Medicine (formerly the Army Medical Museum), in Washington, DC, requesting permission to study DNA in blood stains, pieces of bone and hair samples from Lincoln. These materials stem from

the evening of his assassination and are preserved in the museum's collection. The museum commissioned a panel to advise it, and last May the panel concluded that there is no legal or ethical impediment to proceeding with the study.

Lincoln has no living descendants, and exhumation is not necessary because, thanks to the polymerase chain reaction, DNA extracted from the museum specimens should suffice. Although perhaps not an issue of great historical significance, the possibility that Lincoln had Marfan syndrome is a question worth answering now that the means of doing so are in sight. A technical panel will meet in November to advise the museum on how the study should be done. Certainly, something approaching the full repertoire of mutations in the fibrillin gene *FBN1* on chromosome 15 and other genes capable of causing the syndrome should be worked out first.

Whether it is an important historical question or not, the renewed interest in the possibility that Lincoln had Marfan syndrome has boosted the self-esteem of Marfan patients and patients with other genetic disorders. In his syndicated column of 22 February 1991, bioethicist and columnist Arthur Caplan expressed the opinion that the project amounts to voyeurism. An angry writer of a letter to the editor pointed out that Caplan seemed to imply that there is something scandalous and disgraceful about Marfan syndrome, and that the

effort to discover it in Lincoln is in effect an *exposé*. She said, "I have the Marfan syndrome. . . . The fact that Lincoln may have had Marfan syndrome shows those of us with genetic syndromes that we too can contribute something of value to society. . . . It's time that all people, especially medical ethicists, realize that having the Marfan syndrome is not shameful — it's just darned inconvenient".

The interest in possible Marfan syndrome in Lincoln may not only help to counteract a prevalent and unfortunate attitude towards genetic disease in general, but is bound to draw attention to Marfan syndrome and increase awareness of it on the part of both the general public and physicians. It can do for the disorder what poliomyelitis in President Roosevelt did for that disease. The vaccine against poliomyelitis certainly would not have been developed by 1955 were it not for Roosevelt's polio and for his founding of the National Foundation for Infantile Paralysis in 1937. Indeed, most of the beginnings of molecular biology were financed by the polio foundation as part of its study of viruses in search for a vaccine. Max Delbrück and Salvador Luria, both Nobel laureates, had that support, as did James Watson when he worked at Cambridge with Francis Crick in 1951–1953.

I would put the chances of Lincoln's having had Marfan syndrome at 50:50. It is not mere curiosity that impels us to try to find the answer one way or the other.

V. McK.

had been labelled Marfan syndrome was identified in the 1960s when homocystinuria, a rare autosomal recessive inborn error of metabolism, was discovered^{16,17}. This disorder, like Marfan syndrome, is accompanied by dislocated lenses, skeletal changes including excess height, and serious cardiovascular problems, but on close examination there are differences in all three of these aspects.

In 1971, Beals and Hecht¹⁸ described an autosomal dominant disorder they called congenital contractural arachnodactyly (CCA). The skeletal features are like those of severe Marfan syndrome but there are joint contractures rather than loose-jointedness and the eye and aorta are not affected. Indeed, Hecht and Beals¹⁹ suggested that Dr Marfan's patient had CCA and not Marfan syndrome. Lee *et al.*² now present linkage data indicating that CCA is caused by mutation in a second fibrillin gene (*FBN2*), located on chromosome 5. They demonstrate that fibrillin is probably a family of connective tissue proteins (comparable, at least on a small scale, to the collagens, of which there are a dozen or more genetically distinct

forms) and that a Marfan-like disorder (CCA) can be caused by mutation elsewhere than in the *FBN1* gene on chromosome 15.

In studies of the 70 per cent of the chromosome 15 fibrillin gene (*FBN1*) cloned to date³, no deletions or other large rearrangements have been found thus far in Marfan patients. It may turn out that most of the changes are point mutations. Both of the unrelated patients with the *de novo* arginine-to-proline mutation identified by Dietz *et al.*⁴ have severe early-onset Marfan syndrome. Experience with mutations in the collagen and other large genes indicates that mutations at different sites in the gene (allelic mutations) can result in a clinical picture that is quantitatively and even qualitatively distinctive. It is plausible, for example, that a mutation in the *FBN1* gene could cause a form of Marfan syndrome like that that may have affected Abraham Lincoln (see box and cover). Moreover, abdominal aortic aneurysm, which increasing evidence points to having a genetic basis in most cases²⁰, and familial aortic dissection without other clear features of Marfan

syndrome, are candidates for fibrillin mutations.

What of the diagnosis and treatment of the syndrome? Studies in the 1950s (refs 1, 5) demonstrated that aortic dissection and rupture do not occur out of the blue, but rather against a background of progressive dilatation of the aorta, and that the first part of the aorta to be affected is the root and ascending portion, within the radiographic shadow of the heart. Considerable asymptomatic dilatation of this portion can occur, leading to aortic regurgitation and even rupture, before aneurysm is discernible by chest radiograph. Since the 1970s, echocardiography (cardiovascular ultrasonography) for noninvasive determination of the size of the root of the aorta has been a great boon in management of patients with the Marfan syndrome. The presence of an aortic root of normal size, although not excluding the syndrome completely, makes it less likely and, at any rate, rules out the main life-threatening feature of the disorder.

As to therapy, in the late 1960s use of β -adrenergic blockers was begun in Marfan patients. The objective was to reduce

the abruptness of ventricular ejection, thereby reducing the physiological pounding on the ascending aorta; these agents are now used fairly routinely in patients who are beginning to show dilatation of the aortic root. And 20 years ago Hugh Bentall introduced the composite graft operation, in which the aorta and aortic valve are replaced by a synthetic tube that has an artificial valve sewn into the proximal end. In its various modifications, the operation has been highly successful in treating the syndrome²¹.

But Marfan syndrome still goes undiagnosed (and therefore untreated). It

also kills, as evidenced most dramatically by the occurrence of sudden death from aortic rupture in athletes such as the volleyball player Flo Hyman²². Definition of the gene lesions responsible for the syndrome should permit the design of definitive diagnostic tests, though the large variety of different mutations may mean the task is by no means straightforward. With the results described here, we therefore witness not the end of the story but the opening of a new chapter. □

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MOLECULAR SIEVES

Grand openings for cloverite

Mark E. Davis

MICROPOROUS crystalline solids comprise a class of materials whose main uses are as adsorbents, catalysts and ion exchangers. Zeolites, the best known of these, are built from tetrahedral units of SiO₄ and AlO₄ which connect to form crystalline structures with cavities and/or channels large enough to accommodate (or host) guest molecules. The degree of structural specificity achievable with molecular sieves is highly advantageous in the synthesis of materials with precise functions, such as optically active materials. On page 320 of this issue¹, Estermann *et al.* report a new molecular sieve, which they call cloverite, which has a supercage of 30 Å diameter, significantly larger than any other known cage. Although it is cloverite's most notable feature, the supercage is only one of several structural aspects that may give the new material unusual catalytic properties.

Cloverite's chemical cousins, the phosphate-based molecular sieves (built from PO₄ and MO₄ tetrahedra where M can be Al, Ga, Zn, Be and so on), have been known for approximately a decade. Normally, these materials are made solely from tetrahedral units, as are zeolites. Therefore, one of the interesting features of cloverite is the presence of terminal hydroxyl groups (PO₃OH and GaO₃OH)

which are an intimate part of the structure. (Because the framework does not fully consist of four connected tetrahedra it is called an interrupted framework.) The 20-membered ring pore of cloverite (the ring number specifies the number of phosphorus and metal atoms that form the ring) includes four terminal hydroxyl groups which intrude into the opening to give it a cloverleaf shape (see figure).

Zeolites with pores consisting of 12-membered rings have been known for many years. But it was only in 1988 that we reported² the first molecular sieve with larger pores, VPI-5. This is an aluminophosphate containing unidimensional, circular pores made of 18-membered rings with free diameters of about 13 Å. Thus, cloverite is unique both in that it has the largest ring size and in that it is the only molecular sieve to have a cloverleaf pore geometry. Although this geometry may exclude from the crystal interior of cloverite certain molecules taken up by VPI-5, the unusual shape may be useful in creating novel shape-induced adsorption properties.

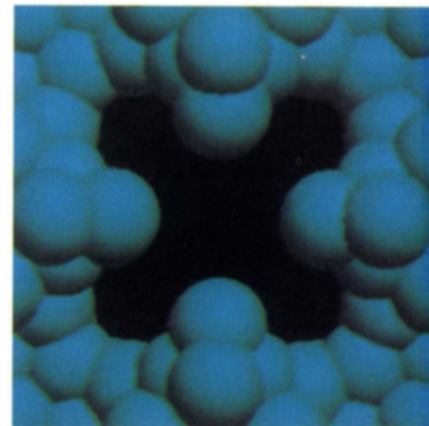
The most exciting feature of cloverite is its 30-Å cage. Each cage can be accessed through six clover-shaped pores, making the material's pore system three-dimensional; VPI-5, by contrast, is uni-

dimensional. As I described previously in News and Views³, multidimensional, large-pore molecular sieves have many advantages. Estermann *et al.* comment on several of cloverite's, so I will concentrate on a specific example — catalyst immobilization — of how cloverite could be used as a host.

Over the past decade or so, zeolites have been used for a kind of ship-in-a-bottle synthesis of organometallic complexes: the individual reagents are small enough to enter the pore system, but the assembled complexes are too large to escape the cages. Such complexes are often highly effective as catalysts, especially in the synthesis of chiral (asymmetric) compounds. The trapping, or immobilization, of the complexes is often desirable because of the expense of their constituent metals and intricate ligands. Until now, the 13-Å cages have limited the range of catalysts that may be immobilized, but cloverite's expanded, 30-Å cage should loosen that restriction. For example, chiral versions could be made in cloverite of the achiral rhodium phosphine complexes we have synthesized⁴ in zeolite X and Y. Such complexes could be used to catalyse asymmetric reactions to form bioactive molecules such as L-DOPA.

Of course, biocatalysts are not the only interesting guests for cloverite. Quantum-sized semiconductor particles — particles so small that quantum-mechanical effects dominate their behaviour — are a new class of materials being closely studied. These could be controllably synthesized in the cages of cloverite as they have been in other microporous solids (see the News and Views article by Grätzel⁵). And although the cage surfaces will not be so different in atomic-scale geometry from those of nonmicroporous catalysts, the combination of the unique pore shape and the large number of terminal hydroxyl groups may endow cloverite with unusual catalytic properties.

The synthetic route to cloverite holds



Lucky for some: the four-leaf pore opening into cloverite's supercage. (Computer image courtesy of C. Baerlocher.)

the promise of interesting future possibilities. The use of fluoride (F^-) appears critical to the synthesis of cloverite, as it remains encapsulated within the material's paired 4-membered rings after the synthesis. Estermann *et al.* report that another gallophosphate with the zeolite A structure (LTA) was also synthesized using F^- . The LTA structure includes a large number of double 4-membered rings and numerous structures can be derived, in principle, using these structural units. So we can expect a plethora of new molecular sieves to follow (an equivalent explosion of activity followed the first synthesis of aluminophosphate molecular sieves).

The recent flourish of new molecular sieves with pore sizes greater than 12-membered rings (VPI-5, $AlPO_4$ (ref. 6) with 14-membered rings, and now cloverite) after years of stagnation at that limit, is probably attributable to the switch from the silica-based syntheses of zeolites and silicates to the phosphate-based structures⁷. The crystallization of the latter (from acidic reaction mixtures, as opposed to the basic ones of the zeolites

and silicates) clearly proceeds in a different way to allow the formation of large rings. Estermann *et al.* comment that a pure silica form of an aluminophosphate (AST) molecular sieve which contains a large number of double 4-membered rings can be synthesized using F^- . Thus, they provide clues on how it may be possible to synthesize the first silica-based molecular sieve with pores consisting of greater than 12-membered rings. A silica analogue of cloverite may in fact be a good candidate, as intracrystalline terminal silanol groups (SiO_3OH) have been observed in significant quantities in other essentially pure silica molecular sieves like ZSM-5. □

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ATMOSPHERIC CHEMISTRY

New ozone hole phenomenon

Anne M. Thompson

OZONE depletion over Antarctica is not restricted to the stratosphere, it seems. Schnell *et al.*¹ have recently reported that as the ozone hole develops in the upper atmosphere, during the Antarctic spring, so also the lower atmosphere, or troposphere, loses ozone. Both photochemical perturbations (the result of increased ultraviolet irradiation attributable to the stratospheric ozone hole) and changes in tropospheric circulation are responsible, the authors suggest.

The ozone hole, which was first identified in 1985, develops in the stratosphere, over a height of 14–22 km (ref. 2), each southern spring as a combination of fresh sunlight, manmade halocarbons and extremely cold temperatures set off chlorine-releasing chemical reactions on ice cloud particles inside the polar vortex. Although the hole disappears as the stratosphere warms and the vortex breaks up, the spring hole has become worse than when it first developed in the mid-late 1970s (ref. 3). One consequence of the ozone hole is ozone loss beyond Antarctica during the austral spring and summer when air masses with chemically induced stratospheric ozone loss penetrate towards mid-latitudes⁴ — a dilution effect. Indeed, a report just published by Stolarski *et al.*⁵ shows significant total ozone loss in both Northern and Southern Hemispheres to within 35° of the Equator.

Now Schnell *et al.*¹ have shown from measurements at the NOAA Monitoring Station at the South Pole that stratospheric ozone depletion may be leading to surface ozone losses during the austral spring and summer. The photochemical explanation is that stratospheric ozone loss admits more ultraviolet radiation into the troposphere which destroys more tropospheric ozone by photodissociation, a two-step process leading to formation of the hydroxyl radical (OH). Two competing effects result: (1) catalytic ozone loss from hydroxyl and

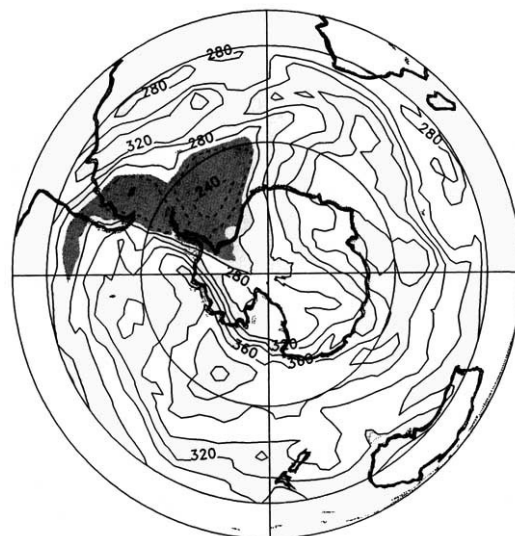
its peroxy radical partner; (2) ozone production from smog reactions with nitrogen oxides and hydrocarbons. In Antarctica, tropospheric ozone losses dominate because concentrations of the ozone formation precursors are too low to make ozone efficiently.

The possibility of tropospheric ozone loss (and formation) due to stratospheric ozone loss has been foreseen in model calculations^{6–8}, but not previously observed. Now given apparently widespread ozone hole dilution⁵ over the Southern Hemisphere, the question becomes — will tropospheric ozone responses to increased ultraviolet irradiation become widespread? Also, because ozone changes in the troposphere frequently lead to higher levels of OH and hydrogen peroxide, there is concern that stratospheric ozone losses could perturb these atmospheric oxidants as well.

On average, total ozone has decreased in high and mid-latitude Southern Hemisphere Decembers by 5–15 per cent over the past decade⁹. Photochemical model calculations^{7,8} show that 10 per cent stratospheric loss over unpolluted regions (like the oceans) would lead to a 5 per cent reduction in total tropospheric ozone (from the ground to 15 km level) and 5–10 per cent gain of the oxidants OH and hydrogen peroxide. These oxidant increases would have mixed effects on the troposphere. On one hand, they lead to the formation of acid rain (not desirable) but healthy levels of OH are needed to suppress levels of the gases that are being adopted as chlorofluorocarbon substitutes.

There is an additional complexity in the effects of ultraviolet on air quality — model calculations^{6–8} also show that in urban areas, stratospheric ozone depletion with an accompanying increase in ultraviolet irradiation is likely to accelerate the development of photochemical ozone smog, in contrast to the destruction expected in the marine environ-

An example of extreme ozone depletion spread across lower South America and surrounding oceanic regions. Polar orthographic projection of Southern Hemisphere ozone from TOMS for 12 December 1987. (Contour interval, 20 Dobson units (DU).) In the shaded region O_3 is less than 270 DU. Underneath this region of low ozone, surface ozone losses from higher ultraviolet levels are twice what they are under normal overhead ozone conditions in a nonpolluted marine environment. In an urban environment under the same air mass, however, ozone production rates could be 5–15 per cent higher than normal. (Courtesy P. Newman, NASA/Goddard.)



ment. Indeed, the Total Ozone Mapping Spectrometer (TOMS) satellite reveals episodes of air depleted in stratospheric ozone persisting for days to weeks over southern Australian and, occasionally, South American cities (see figure) in late spring and early summer after the ozone hole breaks up.

Schnell *et al.*¹ also believe that changing transport patterns near the South Pole are an important factor for the summertime Antarctic tropospheric ozone decrease. It seems premature to conclude from their observations of a temporal increase in cloudiness and increased advection of lower-latitude ozone-poor air whether there is a trend in polar dynamical patterns or not. Schnell *et al.*¹ also rule out injection of ozone-poor stratospheric air as a cause of reduced tropospheric ozone at South Pole, citing their McMurdo (76° S) ozonesonde record, but does the South Pole record⁹ support the same conclusion? It appears that further analysis of polar dynamics is required before the hypothesis of altered transport patterns can be accepted.

The possibilities that Schnell *et al.*¹ have raised for perturbed ozone on a larger scale make one wonder whether losses in tropospheric ozone are detectable elsewhere in the Southern Hemisphere. Suitable surface and balloon ozone measurements should be checked. Detection of ozone changes responding to perturbed ultraviolet in urban locations might be possible, but distinguishing trends due to stratospheric ozone depletion from increases in pollutant emissions is probably not.

To determine whether the changes detected by Schnell *et al.*¹ are truly large-scale will require global analysis. The work of Fishman and coworkers¹⁰, who have invented a method for obtaining mid-latitude tropospheric ozone abundances from satellite data, might be adapted for higher latitudes. □

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Splicing: the new edition

Brenda L. Bass

EXPRESSION of certain mitochondrial genes in kinetoplastid protozoa requires a post-transcriptional event called RNA editing (reviewed in refs 1 and 2). During editing, nascent transcripts that lack a functional open reading frame are converted into translatable messages by

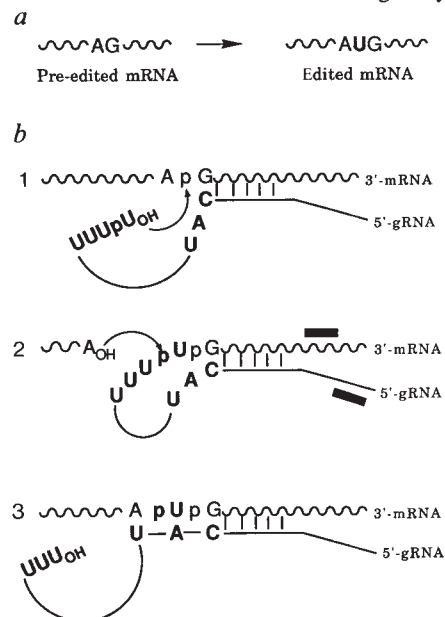


FIG. 1 Transesterification mechanism for RNA editing. *a*, The overall reaction; *b*, the mechanism by which it occurs. gRNA sequences are shown in bold print. Phosphates (p) are shown only for those phosphodiester bonds involved in the transesterification reactions. Black bars denote the primers used in PCR.

the insertion and deletion of uridine residues. A report in *Cell* by Blum *et al.*³ provides an elegant solution to two of the many mysteries surrounding RNA editing: the source of the nonencoded uridines found in the edited transcripts and the mechanism by which uridines are inserted and deleted. By characterizing molecules thought to be intermediates in the editing process, the authors have come up with compelling evidence that the uridines derive from the 3' termini of the recently described guide RNAs (gRNAs)⁴, and that the mechanism of uridine insertion and deletion can be explained by a series of transesterification reactions.

A model for the mechanism of RNA editing formed the basis of the investigation and is shown for a single uridine insertion in Fig. 1 (see ref. 5 for a more complete discussion of the model). The idea is that each uridine to be inserted or deleted during editing can be thought of as a tiny intron and thus, like many forms of RNA splicing, the requisite cleavage and ligation events would occur

by way of a series of transesterification reactions⁶. Although uridine deletion would be most analogous to intron excision, uridine insertion could be thought of as the reverse reaction. A key feature of the model is the participation of the nonencoded uridines found at the 3' termini of gRNA molecules⁷ (gRNAs are small mitochondrion-encoded RNA molecules that are complementary to short regions of fully edited messenger RNAs). Although not yet proven, the nonencoded 3'oligo(U) tail found on these molecules is thought to be added by a mitochondrial uridylyltransferase activity⁸.

As in previously proposed models⁹, Fig. 1 shows a gRNA base-pairing, downstream of the editing site, which serves to direct the editing process to the first mismatched nucleotide. The overall reaction requires two transesterification reactions, the terminal uridine of the gRNA serving as the nucleophile in the first, and thus leading to its incorporation into the mRNA. For a deletion, the initial nucleophilic attack would occur 5' rather than 3' of the mismatched residue, which in this case would be the uridine to be deleted. The subsequent attack by the 3' hydroxyl of the mRNA would occur 3' of the uridine resulting in its excision and a gRNA that was one nucleotide longer.

The model predicts a reaction intermediate in which the gRNA is covalently linked to the mRNA (see step 2 in Fig. 1). To search for such gRNA-mRNA chimaeric molecules Blum and colleagues performed a polymerase chain reaction (PCR) with mitochondrial RNA, using a 5' primer specific to the gRNA and a 3' primer specific to the mRNA (step 2). The exciting (yet predicted) result was that chimaeric molecules were detected for three different genes of *Leishmania tarentolae*: NADH dehydrogenase subunit 7 (ND7), and subunits II and III of cytochrome oxidase. The authors carried out control reactions to rule out artefacts caused by 'jumping PCR' and to show that the generation of chimaeric molecules was primer specific; their results thus provide strong support for the splicing model as well as the role of the gRNA in donating uridines.

The PCR products were cloned and sequenced. For each gene analysed, Blum *et al.* obtained a number of chimaeric sequences representing insertion or deletion intermediates for the different editing sites of the various mRNAs. A representative sequence for the junction of an ND7 gRNA-mRNA chimaeric

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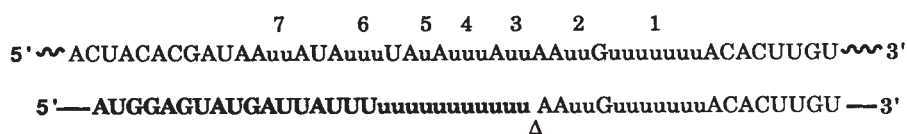


FIG. 2 The junction sequence of a gRNA-mRNA chimaeric molecule is shown below the fully edited mRNA sequence. Non-encoded uridines are shown in small script. gRNA sequences are shown in bold, and the editing sites are numbered from 3' to 5' in the fully edited molecule. Δ marks the site of gRNA attachment to the mRNA.

molecule is shown in Fig. 2. According to the model, the molecule shown arose during the addition of the first uridine of the third editing site. Sites one and two are fully edited, which is consistent with previous work that suggests editing proceeds in a 3' to 5' direction¹⁰.

Not all of the molecules sequenced fit into the precise and orderly pattern of editing exemplified by the molecule in Fig. 2. In fact, in a few cases, gRNA sequences were attached at sites that do not require editing to produce the mature mRNA. Similar aberrant editing patterns have been observed in studies of partially edited mRNAs^{11,12}, and have led to the hypothesis that editing is an inherently imprecise process requiring several rounds before the correct molecule is formed¹¹. Alternatively, it has been proposed that in some cases errors occur due to editing that was guided by the wrong gRNA or hybridization of the correct gRNA to the wrong site¹². Although the idea that functional mRNAs may be generated by trial-and-error is a bit disconcerting, at present this possibility cannot be excluded.

Some of the splicing reactions that occur by transesterification, for instance those involving group 1 introns¹³, can take place in the complete absence of protein, the intron acting as the biological catalyst or ribozyme. Others, such as splicing of nuclear mRNA, require proteins, but may be mediated by RNA catalysis¹⁴. In this case the proteins would not be directly involved in catalysis but would serve to promote the proper RNA structure and the fidelity of the reaction. As Blum *et al.* point out, it is possible that the gRNAs themselves are ribozymes. If proteins are also required, perhaps their function is to provide specificity to a ribozyme that in the absence of protein will randomly insert

and delete uridines into pre-existing transcripts. This idea raises the possibility that the unexpected editing patterns are generated during the isolation of the RNA—that is, once the proteins are removed the ribozymes would be free to react randomly to yield aberrantly edited molecules.

Although the authors³ put forward alternative models for the production of the chimaeric molecules, the sequences of the molecules are so consistent with the predictions of the transesterification model that it seems unlikely to be coincidental. *Aficionados* of RNA processing

will find the new model particularly attractive. While previously proposed mechanisms for RNA editing were cumbersome, requiring the successive action of an endonuclease, a uridylyl-transferase and an RNA ligase, the new model invokes a series of reactions that could occur in a single active site⁵, and furthermore uses catalytic mechanisms and strategies known to be involved in other RNA-processing reactions.

Those with a desire to enlarge our picture of a prebiotic RNA world will of course raise the unanswerable question as to whether RNA editing was the predecessor of modern-day splicing. Those wanting conclusive evidence about the mechanism of RNA editing will merely have to wait for the development of an *in vitro* RNA-editing system. That wait is likely to be a short one. □

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MOTOR PROTEINS

Biomechanics goes quantum

Malcolm Irving

MANY motor proteins use the free energy of ATP hydrolysis to produce force or motion on a polymer track, the best characterized example being the myosin motor which moves actin filaments in muscle. The energy input to each motor is quantized in units corresponding to hydrolysis of one ATP molecule. High-resolution measurements on small numbers of motor molecules now make it possible to observe quantization of force and motion directly, and to estimate the unitary motor force and the distance moved for each ATP hydrolysed. Thus on pages 301 and 307 of this issue, Ishijima *et al.*¹ (Osaka University) and Uyeda *et al.*² (Stanford) respectively describe the force fluctuations in a single actin filament and quantization of actin filament velocities. The distance moved per ATP hydrolysed has also been measured in more physiological conditions in muscle fibres, as reported by Higuchi and Goldman³ on page 352.

The myosin motor is a versatile machine which can produce force and motion in a variable ratio, much like a simple motor with an automatic gearbox. Inertial and viscous forces on an actin filament are negligible compared with the unitary motor force. In the absence of any external force, intrinsic motor properties determine a maximum velocity of filament sliding of several micrometres a second. This maximum velocity is related to the unitary motor step, which can be defined in two ways. The 'power stroke' of the motor, like the

cylinder stroke in a car engine, is the unitary structural change in the motor mechanism. From mechanical measurements on muscle⁴, it seems that the power stroke is about 10 nm; this is plausible for a structural change in the active motor domain of myosin — the myosin head — which is about 20 nm long. The other definition of unit step, the distance moved for each ATP molecule hydrolysed, is more closely analogous to fuel economy. But the two quantities would be equal in the simplest tight-coupling model in which, for each ATP molecule hydrolysed, the motor binds once to actin, executes one power stroke, then detaches.

In vitro studies

In the *in vitro* studies of the motor mechanism described here^{1,2} and elsewhere^{5,6}, myosin is bound to a flat surface and fluorescently labelled actin filaments slide over the surface when ATP is added. The Osaka group¹ has now measured the force on a single actin filament with remarkable sensitivity, by attaching the filament to a glass micro-needle of known stiffness and monitoring the needle deflection in the nanometre range. If the needle is relatively stiff and only a few myosins are interacting with the filament, force is generated with little filament motion, as in an isometric muscle. The force in the filament shows large fluctuations, with amplitude and frequency characteristics expected from random, independent

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transitions between a high-force (on) and zero-force (off) state in the myosin motors. The force in the on state, assumed to be a constant in this simple model, is about 1 piconewton. The rate of transitions between these two states matches the rate of ATP hydrolysis, as expected in the simple tight-coupling model.

If a greater length of actin filament interacts with the myosin-coated surface or the needle is less stiff, the filament may slide at almost constant velocity, as in active shortening of muscle. At near-maximum velocity, the amplitude of the force fluctuations is very small. Interpreting these results with the simple on-off model implies that each myosin stays on for almost the whole period of each ATP hydrolysis cycle, during which the filament slides by more than 100 nm, a distance much greater than the conventional 10 nm power stroke. But this conclusion may depend on the use of the simple on-off model, which cannot account for many of the mechanical properties of muscle. These properties can be explained by postulating an elastic element and a power stroke within the attached myosin head^{4,7}, and it may be useful to analyse the *in vitro* data in terms of these more complex models. The Osaka group¹ have already embarked on such an analysis for the isometric case.

The results from Uyeda *et al.*² complement those of Ishijima *et al.*¹ in showing that filament velocity is also quantized. Actin filament sliding on surfaces coated with a very low density of myosin heads has been characterized at high resolution. Filament velocity decreases with decreasing myosin density or filament length⁵, suggesting that velocity depends on the number of actin-myosin interactions. This in turn implies that each myosin actively pulls on the actin filament (executes a power stroke) for only a small fraction of the duration of each ATP hydrolysis cycle. This fraction, called the duty ratio, has been estimated⁵ as 0.05. To emphasize the similarity with the on-off model used by the Osaka group, I will refer to this model as the go-stop model. Both models assume random independent action of the motors with a fixed quantum of force or movement. Both omit the elastic character of the motor which is a fundamental element of conventional muscle models⁷.

In the go-stop model, when only a few myosins pull on a filament, their contributions to filament velocity effectively add, because it is unlikely that two pull at the same instant. If only one myosin can interact with the filament, its velocity should be the maximum sliding velocity multiplied by the duty ratio. Two myosins should give roughly twice this velocity, and so on. In their new work,

the Stanford group² has directly observed the predicted quantization of velocity. Multiplying the unitary velocity by the ATP hydrolysis cycle time gives a sliding distance of about 10 nm for each molecule of ATP hydrolysed. This value is equal to the conventional power stroke, as expected in the tight-coupling model.

Passenger interaction

However in the long waiting period between power strokes the motor does not detach from the filament, but continues to interact with it and suppress its brownian motion². When many motors interact with one filament so that it slides at maximum velocity, the total sliding distance during the time each motor hydrolyses one ATP is about 10 nm/0.05, giving a value of 200 nm. This distance is made up of the 10 nm active power stroke, plus a much larger distance over which the motor is effectively a passenger. When many motors interact with a filament, the total sliding distance per ATP molecule hydrolysed over which a given motor interacts with the filament, either as locomotive or passenger, could be almost 200 nm. This interaction distance is much bigger than the length of the myosin head, implying multiple detachment/attachment cycles in the passenger interaction, in contrast with the simplest form of the tight-coupling model.

Both the power stroke and the interaction distance can also be measured in intact muscle fibres in which some of the constraints and uncertainties of these *in vitro* systems do not apply. Many myosin motors and actin filaments are involved in the motion, so the unitary action of a single myosin cannot be observed directly. Nevertheless it is possible to estimate the fraction of myosins that are interacting at any instant by measuring the stiffness of the muscle. During filament sliding at maximum velocity, these measurements^{3,8} suggest that the interacting fraction is about 40 per cent. Higuchi and Goldman³ have now measured the filament sliding produced by liberation of small amounts of ATP in muscle fibres and show that the sliding distance is proportional to the amount of ATP released. At near-maximum sliding velocity, the interaction distance (assuming that 40 per cent of myosins are interacting at any instant) is estimated to be at least 40 nm and probably larger. A similar calculation can be made from the steady state ATP turnover rate in intact muscles, which is five per second for each myosin head during filament sliding at $2 \mu\text{m s}^{-1}$ (ref. 9). With 40 per cent of the heads interacting at any instant⁸, the interaction distance estimated from these data is 160 nm.

Within the uncertainties of the experi-

Tunnelling in

THE first tourists to emerge from the British end of the channel tunnel were not Frenchmen in search of warm beer and boiled mutton served with mint sauce, but spiders. The linyphiid spider *Minicia marginella* (Wider) is common in northern Europe, but was unknown in Britain until R. Snazell collected specimens from the British end of the Shakespeare Cliff tunnel workings near Dover, just 5 metres above the shingle beach (*J. Zool., Lond.* **224**, 381–384; 1991). Home Office officials will be interested to learn that the spiders could be permanent immigrants rather than day-trippers: two were found, a male and a female.

Do it yourself

FREE lunches are on the agenda again for high-temperature superconductors. Already H. Matsuzawa and colleagues have demonstrated that electron beams passing through a ring of superconductor will focus themselves to a point. Now, they show (*Appl. Phys. Lett.* **59**, 141–142; 1991) that a suitably shaped tube of superconductor could be used to replace the bulky 'wiggler' magnets of synchrotron radiation sources and free-electron lasers. In each case, the twin principles at work are that the moving electrons generate magnetic fields, and that magnetic fields cannot penetrate superconductors. The back reaction of an electron beam's magnetic halo steers it away from the superconductor surface, so that the electrons can be led around bends like light in an optical fibre. So far, Matsuzawa *et al.* have demonstrated merely that the sinusoidal surface of their shaped superconductor does wiggle relatively low-energy electron beams. But they argue that ultimately such devices will be cheaper and easier to use than the permanent magnets currently used in radiation sources.

Cancer confirmation

A STUDY from S. A. Narod *et al.* (*The Lancet* **338**, 82–83; 1991) provides confirmation of last year's linkage mapping of an early-onset breast cancer locus to the long arm of chromosome 17. In three out of five large families with hereditary breast-ovarian cancer, positive evidence for linkage to the same part of the chromosome was found, although it is still too soon to know if the ovarian tumours in these families are due to mutations within the same gene. The work is the first separate confirmation of a positive linkage for a complex clinically heterogeneous disorder, and contrasts with the disappointing mapping results for disorders such as schizophrenia and manic depression.

ments and of these simple calculations, it seems that the *in vitro* data from the Stanford group^{2,5} and the muscle data^{3,4,8,9} are consistent with a power stroke of about 10 nm, and an interaction distance in the presence of many motors which is an order of magnitude larger. The Osaka group has reported⁶ filament sliding of 100 nm or larger per ATP hydrolysed for very short (40 nm) actin filaments moving at maximum velocity on densely coated myosin surfaces. In these conditions the maximum velocity seems to be produced by only one or two myosins per filament, which implies a single power stroke much larger than 10 nm or multiple 10 nm power strokes per ATP hydrolysed. These results and their interpretation appear to be inconsistent with those of the Stanford group. The discrepancies and their possible causes have been reviewed¹⁰, and their resolution probably requires further experiments.

Motor mechanism

Setting this issue aside, what implications do the new results¹⁻³ have for our ideas about the motor mechanism? Direct observation of force fluctuations at the piconewton level and of velocity quantization provide dramatic and powerful support for the conventional view of a motor which attaches to actin, executes a power stroke, and detaches. This action appears to be tightly coupled to ATP hydrolysis in a 1:1 fashion, at least if force generation occurs with little filament motion. When filament sliding is rapid, myosin heads seem to be able to interact with actin over a distance much greater than the conventional 10 nm power stroke for each ATP hydrolysed, at least when many motors act on one filament. The functional and theoretical significance of this extended interaction is not yet clear. Given the experimental discrepancies¹⁰, the extended interaction might involve either multiple power strokes per ATP or passenger myosins which contribute to muscle stiffness. Both possibilities represent a departure from the simplest tight-coupling model during rapid sliding.

The new results on quantization of motor force and velocity have under-

standably been interpreted initially in terms of simple models (on-off or go-stop), analogous to the open-closed models used in conceptually similar measurements on ion channels in membranes. It already seems likely that these models will prove inadequate to describe the function of a motor such as myosin which needs to perform efficiently over a wide range of force:velocity ratios, and the 'quanta' of unit force or displace-

STELLAR EVOLUTION

Collisions and mergers

James M. Nemec

BLUE stragglers are hot, luminous stars found in star clusters of all ages, including the oldest members of our Galaxy, the globular clusters. Although all the stars in a globular cluster are generally thought to have been formed at the same time about 15×10^9 yr ago, the blue stragglers seem to be much younger, only $1-7 \times 10^9$ yr old. On page 297 of this issue¹, Paresce *et al.* report the discovery of 20 blue stragglers in the core of 47 Tucanae, one of the densest globular clusters known. Their results reinforce the belief that the stars were formed from stellar collisions, possibly followed by a merging of the component stars.

The origin and evolutionary history of the blue stragglers in globular clusters has puzzled astronomers for almost 40 years. Part of the trouble has been that very little is known about the blue stragglers (and stellar populations in general) in the centres of the densest clusters, mainly because of difficulty in resolving individual low-luminosity stars in the crowded cores of these clusters. Although blue stragglers are relatively luminous and therefore easy to detect, only 200 have been observed in 15-20 low-density clusters and 100 in the peripheries of a few of the denser clusters.

There is one notable exception. Last year, Aurière *et al.*² discovered several anomalously bright blue stragglers in the centre of the post-core-collapse globular cluster NGC6397, which is a moderately dense system with a central density of 16,000 solar masses ($M_{\odot}$) per cubic parsec. (The solar neighbourhood, in contrast, has a density of $0.065 M_{\odot} \text{ pc}^{-3}$.) The core of 47 Tuc, where Paresce *et al.* have now identified 20 blue stragglers, is even more dense, about $10^5 M_{\odot} \text{ pc}^{-3}$. Not only can these observations help elucidate the puzzle of the formation and evolution of blue stragglers, but as probes of the deep interior of globular clusters they provide new evidence of stellar encounters and mergers that influence, through energy exchange, the dynamical evolution of the cluster as a whole³.

ment may in fact be variable quantities. Fortunately the theoretical framework required to treat them as such has already been developed^{4,7} to explain the properties of muscle, and is applicable to the *in vitro* studies of the myosin motor. □

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Three main mechanisms have been proposed to explain the formation of blue stragglers in globular clusters. First, they may be primordial (15×10^9 yr old) single stars of mass $1-1.5 M_{\odot}$ that have had their lifetimes extended as a result of large-scale mixing currents throughout the star⁴. Second, they may be mass-transfer binary systems⁵ whose components are destined to coalesce into single stars either through angular momentum losses or when one of the components, having exhausted the hydrogen in its core, expands into the red-giant phase. Third, they may be binary systems formed as a result of stellar collisions involving single stars or binary systems^{6,7}.

The first hypothesis (mixing) is attractive in the sense that only modest amounts of fresh nuclear fuel are needed to achieve a considerable extension of lifetime; its main drawback is that the force driving the mixing (perhaps rapid rotation, strong magnetic fields or tidal effects) has not yet been identified. However, the last two mechanisms seem to be the most likely explanations for blue stragglers in globular clusters. Then the question is whether the binaries are primordial or were formed via three-body encounters or tidal capture in two-body encounters.

For low-density globular clusters (and other low-density systems such as old open clusters and dwarf spheroidal galaxies) it now seems clear that the mass-transfer hypothesis, leading to stellar coalescence, is responsible for a considerable fraction of the blue stragglers. In 1988, we found⁸ two of the 50 stragglers in NGC5466 to be contact (W UMa) binary systems, and a third to be a semi-detached or detached binary with an orbital period of half a day. From this, we estimated that 3-15 per cent of the blue stragglers in low-density globular clusters are mass-transfer binaries. As the components of the W UMa systems in NGC5466 are in direct contact, continuously interacting and exchanging mass, and they are bound suffi-

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ciently tightly that it is unlikely that they will be collisionally disrupted, the component stars almost certainly will eventually merge into a single well-mixed star. Also, given that the timescale for such stellar coalescence is less than the blue-straggler lifetime ($1\text{--}7 \times 10^9$ yr) it follows that many, if not all, of the other blue stragglers in NGC5466 may be coalesced binary systems. Indeed the populations of blue stragglers in both NGC5466 and NGC5053 are more centrally concentrated than those of red giants of the same luminosity, which has been attributed to differences in their masses — on average 1.2 and $0.75 M_{\odot}$, respectively. Pulsationally unstable blue stragglers in the ‘Cepheid instability strip’ (SX Phe stars) are also found to be relatively massive.

Segregation

In the case of 47 Tuc, the strong radial gradient in the number density of blue stragglers is further evidence of mass segregation, indicating that these stars too are more massive than the general population. In this sense they resemble those in NGC5466 and NGC5053. But were they formed by the same mechanism? Because of the extremely high density of stars in the centre of 47 Tuc, it would be surprising if the blue stragglers identified by Paresce *et al.* were not formed as a result of stellar collisions. According to Hills and Day⁶, about 1,600 binaries should have formed in 47 Tuc over its lifetime as a result of interactions between single main-sequence stars. Such single-single collisions would produce merged stars which would tend to have a smaller velocity dispersion than the background population and so become centrally concentrated, as is observed with the blue stragglers. It has also been argued⁹ that any primordial binaries in the core of 47 Tuc would have been broken up during the cluster’s 15×10^9 -yr history.

On the other hand, less than one single-single collision is predicted to have occurred in the low-density clusters NGC5053 and NGC5466. For this reason, the blue stragglers in these low-density clusters are more likely to be primordial binaries, or to have been formed as a result of binary–binary interactions which should form new binaries more efficiently than single-single collisions, regardless of the system density¹⁰. The conclusion of Paresce *et al.* is that the blue stragglers in 47 Tuc probably formed through stellar collisions “between single stars and binaries, or between two binaries”, followed by coalescence and subsequent settling to the centre of the cluster. It remains to be seen which of single-single, single–binary and binary–binary collisions is the dominant mechanism.

The recent discoveries^{1,2} of blue stragglers in the cores of the dense clusters 47 Tuc and NGC6397 complement the discoveries within the past decade of cataclysmic variables and millisecond pulsars (both of which are binary systems) and low-mass X-ray binaries in the cores of the highest-density globular clusters. Clearly the rich diversity of binary types that are present depends on the evolutionary histories of the stars as well as the dynamical histories of the clusters, and as such all of these systems provide an invaluable record of the past activity of the parent cluster.

It will be important to follow up the new results with further observations to establish the blue stragglers’ physical characteristics, in particular identifying possible eclipsing binaries, SX Phe stars and spectroscopically peculiar stars among them, for comparison with their counterparts in the low-density globular clusters. In addition, more fields will have to be observed in 47 Tuc to determine whether the distribution of its blue stragglers is centrally peaked or falls off gradually. And of course, the cores of even-denser globular clusters, such as the relatively nearby systems M30 and M15, have yet to reveal their blue stragglers.

Finally, a critical test of the mixing hypothesis was recently proposed¹¹ for the blue stragglers in old open clusters. This test would be equally applicable to those in globular clusters. The isotope ⁷Li is quickly destroyed at temperatures exceeding 2×10^6 K, a temperature easily achieved in stellar interiors. If the blue stragglers are 15×10^9 -yr-old single stars of mass $1.2 M_{\odot}$, whose lifetimes have been extended by mixing, lithium-depleted material would have been brought up to the stellar surface. The detection of a strong neutral lithium line at a wavelength of 670.7 nm in the absorption spectra of blue stragglers would suggest that mixing is not at work. But the apparent faintness of even the brightest globular-cluster blue stragglers precludes that test being made yet. □

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Eyes right!

ABOUT half of us need spectacles. How absurd that evolution should produce an utter marvel like the human eye, and then set the focus wrongly, or get the lens distorted. Daedalus is now fighting back.

He has been inspired by the ‘adaptive optics’ of modern telescopes, whose mirrors are constantly tweaked to correct the distortions of atmospheric turbulence. His novel adaptive spectacle lens is soft and flexible (like the eye lens itself), with surfaces made of the piezoelectric polymer PVDF. Each surface is coated with a pattern of transparent tin-oxide electrodes. By applying a suitable voltage distribution to the electrodes, the lens can be set to any focal length and formed into any optical figure: spherical, cylindrical, whatever.

DREADCO’s Mk I “Adaptaspecs” have a little battery-powered voltage generator on the frame, to define the form of the lenses. An optician testing your eyes doesn’t need to prescribe new lenses, even if your vision has altered. He merely adjusts the voltage settings to their new optima. So one mass-produced pair of Adaptaspecs will fit anyone, and last a lifetime.

Adaptaspecs Mk II are more cunning still. They conduct a sort of continuous eye test all the time. A small infrared diode or laser on the frame projects a known pattern onto the scene being viewed. Your eyes focus this along with the rest of the scene, and form an invisible infrared image on each retina. Now the retina can be examined through the pupil by an ophthalmoscope. So these Adaptaspecs peer into each pupil via a little angled mirror, transparent in the visible but bloomed to ‘half-silvering’ in the infrared. They filter the infrared image from visible light, and observe its shape and focus. Since the exact form of the projected pattern is already known, simple adaptive optimization can tweak the lenses to bring each infrared image to sharp and undistorted perfection. The visible image superimposed on it will be optimized as well. Not only are refractive errors perfectly and continuously corrected: focusing is powerfully aided too. The wearer will find his marvellous new vision maintained even in extreme close-up. Distant scenes, by returning no infrared, will invoke a stored or derived ‘infinity’ setting.

Adaptaspecs seem sure of a wide and grateful market. Daedalus even plans to make special models for cats, dogs, cows and other animals. Their eyes may be as bad as ours, but how to find out? If Adaptaspecs give them fresh vigour, confidence, and interest in their surroundings, the answer will be clear to see.

DAVID JONES

Leukaemia risks and radon

SIR — A correlation has been established between domestic radon exposure and mutation in peripheral T lymphocytes¹. Some caution must be exercised, however, in interpreting this result

The relative risk of lymphoproliferative disease correlates with the same factors that determine domestic radon levels at the county level.

Putative relationships between domes-

SOCIOECONOMIC VARIABLES AND DOMESTIC RADON LEVELS

	Spearman's rank correlation coefficients			
	Radon (arithmetic mean)		Lymphoproliferative disease (relative risk)	
	r_s	P	r_s	P
Unemployment	-0.44	(<0.025)	-0.73	(<0.0005)
Car ownership	0.54	(=0.005)	0.37	(<0.05)
Overcrowding	-0.29	(NS)	-0.25	(NS)
Home ownership	0.11	(NS)	0.04	(NS)
Class I/II	0.42	(<0.05)	0.43	(<0.025)
Class IV/V	-0.41	(<0.05)	-0.44	(<0.025)

Socioeconomic variables from ref. 6; data from the 22 countries examined in ref. 7. Note that radon levels are both positively and negatively correlated with socioeconomic status.

as evidence that levels of domestically encountered radon are sufficient to cause leukaemogenic chromosomal alterations. Radon may simply be acting as a surrogate for some other mutagenic factor.

Levels of radon in houses in the United Kingdom, although strongly related to local geology, are also positively influenced by the quality and value of housing stock². Detached houses have higher radon levels than semidetached or terraced houses which, in turn, have higher levels than flats and maisonettes². The possibility that higher domestic radon levels merely reflect higher socioeconomic status is of concern as such status is an independent risk factor for lymphoproliferative disease³. Domestic radon as a surrogate for socioeconomic status could also confound estimates of lung-cancer risk due to environmental radiation exposure⁴.

Higher radon levels are significantly correlated with the county percentage level of unemployment, percentage of households owning a car, the percentage of households headed by individuals in occupational class I or II as well as the percentage of households headed by individuals in occupational class IV or V (see table). Lower domestic radon levels thus appear to reflect relatively greater socioeconomic deprivation whereas higher levels reflect greater prosperity.

Radon exposure and cancer thus need to be controlled for socioeconomic status and associated factors, at least at the county level⁵. (The correlations may not apply to smaller areas.) Similarly, the causative factors underlying the relationships between higher regional socioeconomic status and leukaemia require closer examination.

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Do bacteria have sex?

SIR — In eukaryotic organisms, sex is the basis of two fundamental phenomena: first, reproduction, and hence maintenance of the species; and second, genetic recombination, and hence generation of the diversity that allows the species to evolve. These different aspects of sex are linked in a single strategy for the maintenance and evolution of the species.

But what about sex in bacteria (J. Maynard Smith *et al.* *Nature* **349**, 29–31; 1991)? The word has been used for three different mechanisms of gene interchange in bacteria, namely transformation, conjugation and transduction, none of which is tied to reproduction, and none involving combination of entire genomes to obtain new individuals. Moreover, they all involve only small specialized pieces of DNA that, in the case of plasmids (conjugation), encode most of the machinery for independent replication, and in the case of phage (transduction) can be considered as bacterial viruses rather than part of the bacterial genome. Furthermore, a usual

rate of plasmid conjugation is 10^{-5} per generation, which is tiny compared with 100 per cent per generation in eukaryotic sexual recombination. In the eukaryotic world, the most similar phenomenon to these mechanisms of bacterial recombination is viral infection, and it seems improper to talk about viral infection in terms of sex.

Why then use the term sex to describe phenomena of genetic recombination in bacteria? Maybe, as pointed out by 'E. Coli' (*Nature* **349**, 97; 1991), our eukaryotic chauvinism makes us think about bacteria from a strongly anthropocentric point of view.

Although at first it seems to be a useful simplification, talking about sex in bacteria is confusing, as we are mixing together completely different concepts. I suggest that the term sex be used to describe only the well-characterized mechanism of reproduction-linked genetic recombination in eukaryotes, leaving bacteria with their own very different mechanisms of genetic recombination, which are completely unrelated to what we usually call sex.

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What's the score?

SIR — Anyone who has participated in the dating ritual knows that a rendezvous does not necessarily mean a 'score'. As Hackett has aptly pointed out in *News and Views*¹, our data² and those of Peters *et al.*³ seem to disagree about the initial site of rendezvous for class II histocompatibility molecules and antigenic fragments. But we do not disagree, in principle, about the timing of the 'score'.

In our paper², in which we reported colocalization of the molecules involved in antigen processing and presentation in early endocytic compartments, we also presented data (Figs 3 and 4) showing that these molecules remain colocalized in endocytic compartments for as long as 30 minutes. We suggested that, although the relevant molecules can intersect early in the endocytic pathway, the gradual acidification and increasing proteolytic activity accompanying maturation in the endocytic pathway could be required for the complete processing of antigen and release of class II molecules from invariant chain. This would then explain the delayed kinetics of antigen presentation relative to the timing of initial colocalization of the molecules involved.

This point may have been missed by those who did not read beyond the title and abstract of our paper. We have gone

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on to suggest that the class II-invariant chain complex is initially targeted to an early endocytic compartment and remains in the endocytic pathway until the class II molecule is released from invariant chain and binds an antigenic peptide⁴. Our observation of mature class II molecules and invariant chain in both early and late endocytic compartments is compatible with dissociation being a gradual process and with the production of most functional antigenic complexes in later endocytic compartments, as proteolytic activity in the endocytic pathway increases. The class II-rich, lysosome-like endocytic compartment described by Peters *et al.*³ could be an endpoint for this progressive maturation of class II molecules, and perhaps the final compartment from which class II molecules can be released.

The discrepancy between our data and those of Peters *et al.* may be due to differences in techniques, cell lines or antibodies used, as suggested by Hackett. But the two studies addressed different questions. We induced formation of endocytic vesicles by internalization of crosslinked immunoglobulin (to mimic antigen uptake) and asked whether immature class II molecules could be found in this induced pathway, without focusing on the overall distribution of class II molecules in the cell, as addressed by Peters *et al.* In our subsequent analyses of the general distribution of class II molecules we have observed, in agreement with Peters *et al.*, that most of the class II molecules are in the trans-Golgi reticulum of the cell, indicating a greater abundance in late endocytic compartments than in early endocytic compartments.

Nevertheless, we remain convinced of the specificity of our observed labelling of invariant chain-class II complexes in early endocytic compartments containing internalized immunoglobulin. In addition to the serological controls published in our original paper², we have now documented that class I molecules in normal cells and invariant chains in class II-negative cells are not localized to such compartments⁴, demonstrating targeting specificity for the class II-invariant chain complex. Invariant chain has also been observed in early endosomes in melanoma cells (G. Griffiths, personal communication). Thus we favour a scheme of gradual maturation of class II molecules within the endocytic pathway which would allow them to acquire different antigenic fragments in different compartments, maximizing the reper-

toire of peptides which can be bound and presented.

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Too exposed?

SIR — We read with interest the Scientific Correspondence by D.K. Felbeck and W.H. Durrant (*Nature* 347, 341; 1990) reporting long strings of repetitive indentations from the surfaces of the long-duration exposure facility (LDEF) exposed to outer space. We have examined all components (clamps, experiment tray lips, intercostals and bolts) of the LDEF, searching for and characterizing features that could be caused by micrometeoroid and space debris impacts. We had previously observed the kinds of features reported by Felbeck and Durrant but had always thought them to be fabrication or handling artefacts. Although great care has been taken in handling LDEF non-experiment components (such as the clamps described below and the experiment tray lips characterized by Felbeck and Durrant) following their return from orbit, handling damage could easily have occurred before flight.

As part of our responsibility as investigators with the LDEF meteoroid and debris special investigation group, we have been examining the chromic-anodized aluminium (6061-T6) clamps which were used to hold experiment trays to the frame of the LDEF. The front face and the edges of these clamps were exposed to space for the entire duration of the 5.7-year orbital mission, but the back face of each clamp was not exposed to the orbital environment. We have now observed hundreds of linear, repetitive-type patterns, of the sort described by Felbeck and Durrant, on both the exposed and unexposed (back)

faces of LDEF clamps. One such feature is shown in the figure. The occurrence of these features on the backs of clamps suggests that all such features are probably fabrication or handling defects, and not due to a previously unrecognized orbital exposure process.

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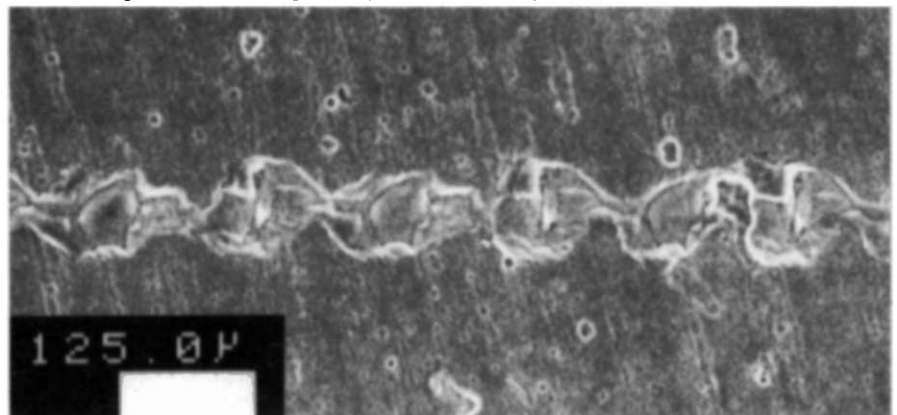
SN2 NASA Johnson Space Center,
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FELBECK REPLIES — We find that two important differences exist between Redd and Zolensky's observations and our own. First, the rough anodized surface of the tray clamps, as can be seen in their figure, severely limits resolution of fine details at high magnification in the electron microscope, in contrast to the remarkably smooth surface of the extruded aluminium-clad strips on which we made our observations. Second, the size of the repeat unit in their example, presumably typical of their observations, is of the order of 250 μm , whereas the size of the repeat unit that we observed on the flight-exposed surfaces is of the order of 20–90 μm in length.

We still find that the few repeat structures we have seen on the protected faces of the aluminium-clad strips are substantially less prevalent and much coarser than those on the flight-exposed surfaces. We are planning a separate study of flight-exposed tray clamps from the LDEF, as well as of a non-flight control clamp. Even after careful examination of commercially available aluminium-clad material similar to our flight strips, we have not observed any repetitive structures of the sort observed on the flight-exposed surfaces from LDEF. In any event, a rational explanation for the source of these indentations is still lacking.

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Scanning electron microscope image of a repetitive linear pattern found on the back (unexposed) side of an aluminium clamp from the LDEF satellite. Scale bar, 125 μm .

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Ball lightning

SIR — Singer in News and Views recently discussed¹ ideas about the origin of ball lightning in the context of Ohtsuki and Ofuruton's report² on fireballs generated in air subjected to microwaves. Singer was wrong to assert that I have concluded that ball lightning is not a plasmoid structure. Quite the contrary. I believe the failure of most plasmoid models is attributable to their use of an invalid single-fluid plasma approximation of the virial theorem. Details of my alternative two-fluid plasmoid model are reported in ref. 3.

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Plastid origins

SIR — We are concerned over the increasingly confused (and confusing) use of the terms monophyletic and polyphyletic in connection with the origin of plastids and mitochondria. The News and Views¹ that accompanied our recent paper² refers to the fact that we show that plastids have polyphyletic origins. Raven³ uses the term polyphyly to mean that plastids in different groups of plants and algae arose, through endosymbiosis, from different prokaryotes with different characteristics.

By comparing eukaryotic-type small subunit ribosomal RNA sequences from a cryptomonad alga, we provide evidence supporting the view that more than one endosymbiotic event has contributed to the diversity of plastids seen today. But we do not interpret our results to mean that plastids have polyphyletic origins. It is quite possible that plastids, having arisen several times as a result of secondary endosymbiotic events, nevertheless had only a single primary (monophyletic) origin⁴. Further comparisons of genes encoded in plastid genomes are needed to decide this issue⁵.

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Similarities in RNA helicases

SIR — There is much excitement about the (putative) RNA helicases from various organisms involved in pre-messenger RNA splicing, ribosomal RNA maturation, translation initiation, ribosome assembly and probably also in other processes related to cell growth and division. A rapidly expanding number of these enzymes, for which the amino-acid sequences have been reported, can be assigned to the previously defined superfamily II, which includes as a subdivision the well-known DEAD family and the putative RNA helicases of several groups of positive-strand RNA viruses. In their discussion¹ of possible functional analogies between the helicases in different systems, Wassarman and Steitz neglected the viral proteins because of the paucity of the evidence relating to their proposed function.

Recently, the RNA helicase and RNA-dependent ATPase activities of one of the proposed RNA viral heli-

cases, the potyvirus CI protein, have been demonstrated^{2,3}. RNA viral helicases and three helicases involved in different steps of yeast pre-mRNA splicing, PRP2, PRP16 and PRP22, share two sequence signatures, DEXH and QxxGRxxR, in the conserved motifs II and VI (ref. 4), as opposed to DEAD and HxxGRxxR in the proteins of the DEAD family⁵.

I have generated a sequence alignment of the entire set of RNA viral helicase belonging to superfamily II, the PRP proteins and those of the DEAD family for the region spanning all seven conserved motifs, and have derived a cluster dendrogram (see figure). The resulting branching order is clear-cut in showing the association between the RNA viral helicases and the PRP proteins.

Whatever the evolutionary bearing of these observations, they indicate that the sequences of the helicases mediating certain steps of pre-mRNA splicing are specifically related to those involved in viral RNA replication. Thus, mechanistic parallels in the helicase action could be even more profound between viral RNA replication and splicing than between splicing and translation, as suggested by Wassarman and Steitz¹. These authors discussed the possibility that the helicases of this superfamily might ensure the fidelity required for splicing and translation.

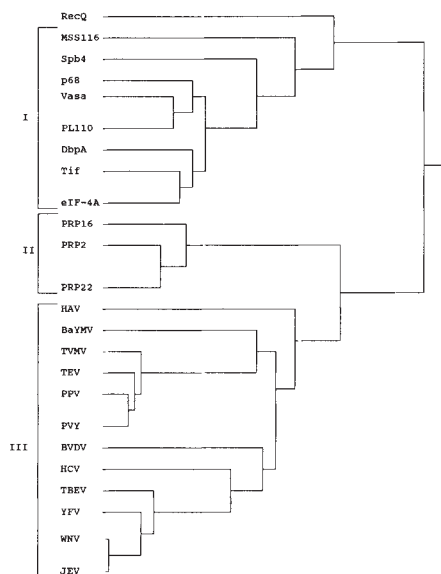
Following this line of reasoning, the helicases of RNA viruses may also reduce the error rate of RNA replication in a manner analogous to that exploited during splicing. This might help to rationalize our previous observation⁶ that relatively large positive-strand RNA viral genomes (greater than five kilobases) all encode the putative RNA helicase, whereas smaller viral genomes lack the respective gene. By reducing the nucleotide misincorporation rate, the helicases may ensure the very possibility of the existence of the larger viral genomes.

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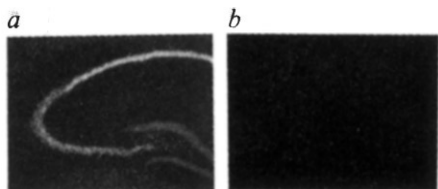


The cluster dendrogram for the cellular and viral helicases was constructed by the UPGMA method⁶. Pairwise distances between the amino-acid sequences were calculated using the method in ref. 7. (Further details of the calculation and the complete list of references are available from the author on request). The groups of proteins are designated: I, DEAD helicases; II, DEAD helicases involved in yeast pre-mRNA splicing; III, RNA viral DEAD helicases. RecQ is an *Escherichia coli* helicase closely related to the DEAD family. BaYMV, barley yellow mosaic virus; TVMV, tobacco vein-mottling virus, PVY, potato virus Y; PPV, plum pox virus; TEV, tobacco etch virus (potyviruses); HCV, hepatitis C virus; TBEV, tick-borne encephalitis virus; YFV, yellow fever virus; WNV, West Nile virus; JEV, Japanese encephalitis virus (flaviviruses); BVDV, bovine viral diarrhoea virus (pestivirus); HAV, *Cryphonectria parasitica* hypovirulence-associated double-stranded RNA virus.

Anti-prions and other agents

SIR — Having observed an open reading frame in the non-coding strand of the PrP gene, Goldgaber in *Scientific Correspondence*¹ predicts the presence of an anti-PrP protein and suggests the use of single-stranded RNA probes to detect expression of this anti-PrP gene.

We have carried out *in situ* hybridization experiments, using ³⁵S labelled single-stranded RNA probes for exon 2 (protein-coding exon) and the large intron of the PrP gene², to examine the expression of the PrP gene in the brain of scrapie-infected and uninfected mice and mouse embryos (our unpublished data). The presence of transcripts from the anti-PrP gene could not be detected by this method (see figure); if such transcripts are present they are at least



In situ hybridization of coronal sections through the brain of VM/DK (*Sinc p7p7*) mice, using ³⁵S-labelled single-stranded RNA probes for exon 2 of PrP. *a*, Antisense and *b*, sense RNA probe. Hybridization was as visualized using nuclear track emulsion and dark-field microscopy.

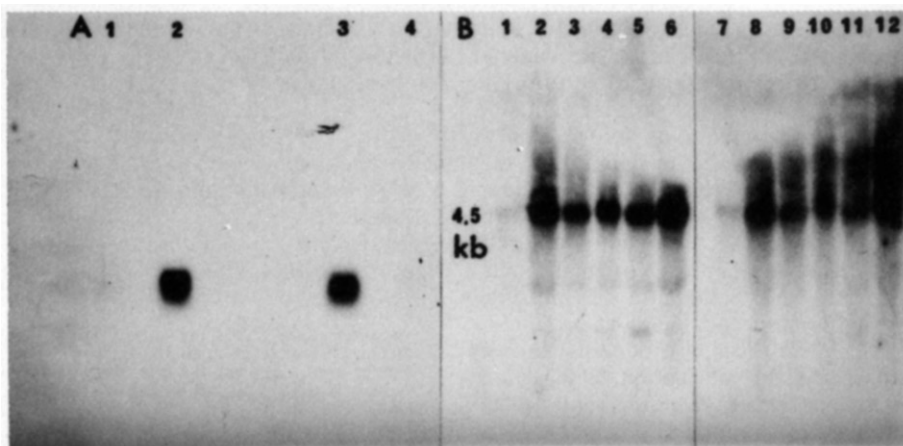
100-fold less abundant than those of PrP. Further search for these transcripts will therefore require the use of more sensitive techniques involving strand-specific RNA amplification. Until then it may be premature to anticipate the anti-prion protein.

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SIR — Goldgaber reported in *Scientific Correspondence*¹ that there is a large open reading frame on the opposite strand of the gene encoding the prion protein (PrP). We had also made this observation and, because of our interest in bovine spongiform encephalopathy, we confirmed the presence of the putative anti-PrP gene in cattle by DNA nucleotide sequencing. To ascertain the expression of this gene, we hybridized single-stranded RNA probes specific to PrP and anti-PrP RNA with total cellular RNA extracted from various tissues



A, Probe specificity; Riboprobes were transcribed from the normal bovine PrP gene under the control of the T3 promoter of Bluescript SK (PrP strand specific) and KS (antisense strand specific). Templates were linearized to prevent erroneous transcription from the T7 promoter. Specificity was ensured by northern blotting unlabelled transcript derived from SK (tracks 1,3) and KS (2,4) strands. Tracks 1 and 2 were hybridized to a radiolabelled SK probe; tracks 3 and 4 were probed with KS. Densitometry of autoradiographs showed no cross hybridization. **B**, Analysis of total cellular RNA; northern-blotted filter contained bovine RNA from brain (1,7), liver (2,8), lung (3,9), mesenteric lymph node (4,10), heart muscle (5,11) and spleen (6,12). Tracks 1-6 were hybridized to SK and 7-12 probed with KS. Exposures were 1h (1-6) and 12 h (7-12), respectively. Loadings of RNA were equivalent with the exception of brain which was approximately 1/10 loading. Washing was to a stringency of $0.1 \times \text{SSC}$ at 65°C .

of a three-week-old calf. All tissues tested were positive with both probes (see figure), providing evidence for the presence of an anti-PrP RNA.

The presence of two mammalian genes transcribed from opposite strands at the same locus is not unique to the PrP gene. Adelman *et al.* reported² that the rate gene encoding gonadotropin-releasing hormone is expressed in the central nervous system whereas a second gene, SH, is transcribed from the opposite strand in heart tissue. We note that in the genes for both PrP and gonadotrophic releasing hormone, the first base of the codon on one strand of DNA corresponds to the third base of the codon on the other strand. This arrangement provides a mechanism for maximum conservation of two proteins and might explain the high degree of interspecies conservation of the PrP gene.

Goldgaber suggests that the expression of the anti-PrP gene introduces "a new player" and presents a different view of spongiform encephalopathies. The hypothesis that two peptides encoded by complementary RNAs would assume conformations that resulted in specific and high-affinity binding of the pair has been postulated for several years³⁻⁵. Consistent with this hypothesis is the fact that the hydrophobicity plot for anti-PrP is almost a mirror image of

that for PrP. If anti-PrP protein is synthesized, it is possible that the two proteins do interact and that disruption of this interaction contributes to the pathogenesis of the transmissible spongiform encephalopathies.

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SIR — New experimental evidence, summarized in *News and Views* by Weissmann¹, makes the protein-only model of the scrapie agent increasingly compelling. Although Weissmann provides a useful summary of some recent data, we would like to clarify the history of the protein-only model for the scrapie agent. Griffith in 1967 proposed² a detailed theoretical model describing a mechanism for the conversion of a normal host protein to a pathogenic form in scrapie. His proposal followed the suggestions by Alper and colleagues³ in 1967 that the scrapie agent may "...replicate without a nucleic acid", and by Pattison and Jones⁴ in the same year that the "scrapie transmissible agent may be, or may be associated with, a small basic protein". On the basis of his earlier work with natural scrapie in sheep, Parry had hypothesized that this disease could occur on a purely genetic basis and be secondarily transmissible⁵.

In 1982 Prusiner introduced the term 'prion' and outlined three mechanisms by which a foreign protein-only agent might replicate⁶; abnormal modification

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of a pre-existing host-protein was not postulated at that time. The scrapie agent protein was subsequently identified⁷ and, to the surprise of many, later demonstrated to be derived from a normal host cellular protein⁸. It is the scrapie isoform, but not the cellular isoform, which is associated with transmissibility⁹. We¹⁰ and others¹¹ have extended Griffith's original model and our view is that the transmissible spongiform encephalopathies are endogenous neurotoxic disorders that can be transmitted by inoculation or ingestion of an abnormally modified form of the normal gene's product, or inherited via a mutation in this gene.

No matter what the transmissible particle is called — agent, virus, virino or prion — it is by all reckoning unique among pathogens. In the prophetic words of Griffith, "...there is no reason to fear that the existence of a protein

agent would cause the whole theoretical structure of molecular biology to come tumbling down".

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Diet and IQ

SIR — One of my coauthors¹ has already responded to Blinkhorn's comments² on our study of the relationship between IQ, vitamin intake and scholastic achievement in four groups of school-children in California³. A more technical response follows.

In our study, three cohorts of children were given vitamin-mineral supplements based on 50, 100 or 200% of the US recommended daily allowances for three months and were compared with a group given placebos. Blinkhorn criticized the finding that supplements improved non-verbal IQ and scholastic achievement, his main point being that 87 tests might have been made and our results could have been due to chance. But only five make-or-break tests were made, one for each hypothesis we formulated.

As predicted, a significant difference in total non-verbal IQ was found ($P=0.007$) on the Wechsler intelligence scale for children—revised (WISC-R) using a four-group ANCOVA of 0 against 50 against 100 against 200 as the make-or-break test on non-verbal IQ. Further examination showed that the 100% cohort caused the four-group ANCOVA to be significant; this cohort did significantly better than the groups taking the placebo ($P<0.001$), the 50% formula ($P<0.05$) and the 200% formula ($P<0.05$). But there was no significant difference when comparing 0 against 50, 0 against 200, or 50 against 200. And, as expected in testing the second hypothesis, no significant difference in total verbal IQ was found on the WISC-R when using a four-group ANCOVA.

A third hypothesis was that if there was a significant difference in non-verbal IQ, the cohort(s) who were responsible might do significantly better than the

placebo group on a scholastic achievement test (the comprehensive test of basic skills, CTBS). Accordingly, the cohort given the 100% formula was compared with the placebo cohort on total CTBS score, which was calculated by summing the 13 subscales and dividing by 13. The 100% cohort produced significantly greater gains ($P=0.024$).

Blinkhorn made two enormous assumptions, resulting in his belief that 87 tests were carried out. First, he assumed that the 23 WISC-R/CTBS subscales were tested, as well as four total scores (full-scale IQ, non-verbal IQ, verbal IQ and total CTBS score). However, only the last three scores were tested — full-scale IQ was not examined at all, and the 23 subscales were studied only to see if the differences were confined to selected areas. (The 100% cohort surpassed the placebo cohort on four out of five of the non-verbal IQ subscales and 13 out of the 13 CTBS scales.)

Blinkhorn's other assumption was that all three of the cohorts given supplements were individually tested against the placebo group for the 23 subscales, four total scores and two group-administered IQ tests (the matrix analogies test (MAT) and Raven's matrices (RM)). This would indeed have produced 87 tests. But in reality, verbal IQ, non-verbal IQ, MAT and RM were each tested on one four-group ANCOVA, while academic achievement was tested on one two-group ANCOVA to give a total of five tests only, not 87.

Blinkhorn also wondered why a change of statistical significance was not expected in non-verbal IQ using the two group-administered tests. The standard deviation on these group tests is about 14 times larger than the expected gain, while the standard deviation of the individually administered WISC-R is about

twice the expected gain⁴.

These tests were used to determine whether non-verbal IQ tests that are administered in a group are reliable enough to pick up slight changes in IQ. The differences on both group tests exceeded the predicted differences, but neither was significant. These results confirmed the need to use individually administered tests, such as the WISC-R, rather than group tests in this type of research.

To assess our claims properly, Blinkhorn felt that presentation of the raw pre- and post-scores was essential. Those tables have now been published, and they show that the groups were not significantly different initially in non-verbal IQ, verbal IQ, CTBS scores, age, sex or race^{4,5}. He also complained that evidence of marked bimodality was not published. Those data, submitted to the *British Medical Journal*, show that the only reason why the 100% cohort differs significantly from the placebo cohort is because 27 of 105 subjects produced a mean gain of 19.6 points rather than the "expected" 6.6 points which would have given the distributions equal means and standard deviations.

It is a pity that most assessments of our study, including Blinkhorn's, have focused on the pros and cons of taking vitamin supplements and have neglected the reason why this research was done. Our previous work showed that when 803 schools implemented diet policies that allowed only nutrient-dense meals, scholastic achievement rose 16% for 1.1 million children⁶. The number of children performing two or more grades below expectations fell from 124,000 to 49,000. A positive correlation between food consumption and achievement occurred after these diet policies were implemented, whereas a negative correlation existed before. We conducted the study because the only way of determining whether better eating habits can have a major effect on performance was to create a placebo-controlled, double-blind trial involving supplements.

My conclusion from our study is straightforward—eat smart to be smart, but if that is not possible a good supplement set at about 100% of the US recommended daily allowances makes sense as an insurance policy. For some children, the results will be spectacular.

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Still poles apart

Derek Fordham

Hero In Disgrace: The True Discoverer of the North Pole, Frederick A. Cook. By Howard S. Abramson. Paragon House: 1991. Pp.249. \$21.95.

"COOK was a liar and a gentleman, Peary was neither", was the opinion expressed by Peter Freuchen, a Danish explorer and trader.

Cook was also an experienced polar traveller when, in an attempt to achieve "the thing that had haunted me for years", he left Anoritoq in North Greenland with ten companions, nine sledges and 103 dogs, a full year before Commander Robert E. Peary. Peary set off in 1909 from the north coast of Ellesmere Island. Both had the North Pole as their objective, although by different routes. Cook, having sent back all but two of his eskimo companions, claimed to reach the Pole on 22 April 1908, but because he was carried far to the west by drifting ice on his return, had to spend a tough winter with his two companions at Cape Sparbo on Devon Island. Having completed an arduous return to Anoritoq in the spring of 1909, he managed to reach civilization ahead of Peary, who maintained he had reached the Pole on 6 April 1909. Cook therefore became the first to have reached 'The Big Nail' — a whole year before Peary.

His claim unleashed against Cook the fury and vilification of the Peary Arctic Club, and the undying opposition of

such formidable bodies as the National Geographic Society, which spared no efforts, fair or foul, to establish the supremacy of their man, Peary.

Publication of a false confession, purporting to be by Cook, admitting that he had not reached the Pole was engineered. Objections were raised to many other aspects of Cook's claim and his earlier ascent of Mount McKinley. He was accused of not being able to supply conclusive proof of his presence at the Pole. Ironical, because this was exactly what Peary was also unable to provide, but in Cook's case it was because Peary had refused to allow Cook's equipment and notes to be brought back from Greenland on his ship.

The continuing warped tirades of Peary's influential backers had their effect and for the rest of Cook's life it was generally held that Peary had reached the Pole and Cook had not. But the one does not necessarily lead to the other. Even though it is now generally

Robert E. Peary — belligerent and opinionated.

accepted by those with the experience to know that Peary did not get closer than about 90 miles to the North Pole, that fact in itself does not imply in any way that Cook did.

Since Cook's death in 1940, serious doubts have been cast on whether he reached the summit of Mount McKinley or the North Pole, and Abramson's decision to omit any serious discussion of most of these doubts diminishes the value of his rather partisan book as a comment on the Cook controversy.

As with Peary's equally disputed claim, no one at this distance in time can know for certain what events took place on the Arctic Ocean in 1908 and 1909. Did Cook press on across the lonely polar ice, or did he, as his companions Itukusuk and Aapilaq innocently state, go only one or two "sleeps" before turning back to Axel Heiberg Island? Did he lie in his tent, as Peary was to do a

year later, and allow the vision of the Pole that had haunted him to become a dream, a dream which then became reality on the pages of his notebook, and a dream which was then to become the nightmare that followed both men to their graves and beyond?

Either way, Cook was an enigmatic and talented man; doctor, ethnologist, designer of polar equipment and an accomplished polar traveller — a man who became embroiled in a world of

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Frederick A. Cook — enigmatic and talented.

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Mary Evans

Mary Evans

Distant goal

Philip Davenport

Fusion: The Search for Endless Energy. By Robin Herman. Cambridge University Press: 1991. Pp. 267. £13.95, \$19.95.

ARISTOTLE specified three essentials for a narrative: a beginning, a middle and an end. These criteria pose problems of balance for an author seeking to tell the story of the quest to exploit thermonuclear reactions as a terrestrial energy source. The beginnings are obscured by a lack of documentation due to the secrecy imposed by governments of the day. By contrast, the period since international agreement on the declassification of fusion research in 1958 has seen a spate of papers, reports, periodicals and comment that must seem indigestible to the lay observer. Finally, it is not yet a success story; an economic fusion reactor is still a distant prospect. Indeed, over the years forecasts of its arrival have tended to recede into the future and are now nudging towards the middle of the twenty-first century.

Robin Herman, a science writer and former reporter, has overcome these difficulties with some panache. By introducing her story with a racy prologue describing a visit to the Joint European Torus (JET) at Culham, England in 1984, she avoids the pitfalls of a strictly chronological format. This sets the scene for a readable and at times exciting account of fusion research from the point of view of a nonspecialist and aimed at the general reader.

Herman's book will inevitably be compared with an earlier one covering similar ground, *Fusion: Science, Politics, and the Invention of a New Energy Source*, by Joan Bromberg (MIT Press, 1982). This is an official history of the US magnetic fusion project and was sponsored by the Department of Energy; it purports to be aimed mainly at those concerned with energy strategies and options. As its title suggests, it comprises a detailed account of the politics and power struggles between officialdom in Washington and fusion laboratories under its control.

In contrast, Herman's book is an entirely private venture stemming from local curiosity; the author lives at Princeton, New Jersey, and became interested in the activities of the Plasma Physics Laboratory there. Receiving a warm welcome, she was encouraged to embark on a national and later global tour of fusion laboratories and to attend the regular international conferences that are a feature of fusion research. The result is a penetrating survey of worldwide progress towards the goal of

economic fusion power, engagingly told using verbatim transcriptions of interviews with many of the scientists and engineers that are prominent in the field.

Over the years, fusion research has suffered from sporadic bouts of sensational reporting in the media, with promises of "limitless power from the oceans" with the aid of "man-made Suns". An early example was the announcement by the Argentine dictator Peron on March 1951, who claimed that a thermonuclear reactor had been developed there by Ronald Richter, an Austrian immigrant. This news was received with informed scepticism by nuclear physicists in both Britain and the United States, a view fully justified by the subsequent events. But the story has been put about, first by Bromberg in her book and now echoed by Herman, that Richter's claim was directly responsible for the inception of the US fusion programme.

Although it may be true that Richter's 1951 claim inspired Lyman Spitzer Jr at Princeton to invent and build his 'Stellarator', the groundwork of fusion research in California which blossomed into the Sherwood project certainly predates it. Serious fusion-oriented work on toroidal gas discharges, led by Peter Thonemann, had begun at the Clarendon Laboratory, Oxford in 1947. James Tuck who went from the Clarendon to the United States in 1949, was fully conversant with this research. He started fusion work at Los Alamos, and indeed the name Sherwood is a reference to his legendary namesake Friar Tuck, who dwelt with Robin Hood in Sherwood Forest. Edward Teller himself visited the Clarendon in 1949. He was shown the work and had animated discussions with Thonemann and his colleagues. Thus there was awareness in the United States of British activity in controlled thermonuclear research some years before the Richter fiasco.

Another example of a fusion 'breakthrough' that was given hysterical publicity by the world media was the cold fusion claim of Martin Fleischmann and Stanley Pons in 1989. Here again, Herman exaggerates its impact on the fusion community. In essence, cold fusion postulated that the metal palladium had magical properties and catalysed the fusion of deuterium, despite the fact that hot palladium has been used routinely since the 1940s for admitting deuterium gas into fusion apparatus without any such effect being detected. One is left wondering why a firm rebuttal of these claims was so long in coming. Perhaps it was delayed by fear of litigation. Lawyers have already bedevilled medical science in the United States; let us hope that physical science will not suffer the same fate.

In many countries, government funding of science is apt to be fickle. In times

of stringency it is subject to cuts, and long-term ventures such as fusion are particularly at risk. Herman believes that the example of JET points the way towards continuity of funding. The existing global collaboration between interested nations should be extended, with enduring commitment, towards full international integration. This somewhat utopian idea could make possible the construction of the huge fusion experiment which many experts believe to be the next logical step. The fusion fraternity should read this book and take heart for the future. □

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Plumbing the depths

A. J. Southward

Deep-Sea Biology: A Natural History of Organisms at the Deep-Sea Floor. By J. D. Gage and P. A. Tyler. Cambridge University Press: 1991. Pp. 504. £80, \$135.

THE bottom of the ocean has been a lure and challenge to scientists for at least 150 years, but is the most difficult part of the biosphere to study. Few of the world's oceanographic vessels are fully fitted to keep station precisely over the sea bed or to handle the large volumes of fine sediments brought up. It says much for Gage and Tyler's perseverance that they have sustained studies of the deep-sea fauna for nearly 20 years, enough to show long-term as well as seasonal interannual variations in the deep-sea fauna and its food supply. They are now giving us a distillation of their own active involvement, set in the context of the past 20 years' worldwide advance in knowledge of the deep sea.

The first section of their book proceeds from a historical summary to a review of the physical environment. Methods of investigation are then described in some detail as far as sampling gear is concerned, particularly infaunal quantitative samplers, epifaunal trawls and remote photographic equipment. Submersibles and remote vehicles are given less attention.

The second section reviews the great diversity of deep-sea life, now known to be far removed from the "abyss... where life is either extinguished, or exhibits but a few sparks to mark its lingering presence" (E. Forbes and R. Godwin-Austen, *The Natural History of the European Seas*, John Van Voorst, London, 1859). There are the megafauna of often strange aspect: the glass-

sponges, the holothurians, giant sea-spiders, scavenging amphipods and isopods, crabs and squat-lobsters, sea fans, tunicates and, of course, the bottom-feeding or hovering fishes, including the ubiquitous macruids as well as specialized forms like the tripod fish. Large sea pens exist, rooted deep in the ooze, and there can be masses of the otherwise extinct sea-lilies. Next comes the ordinary-sized macrofauna, including many burrowing species, and finally the meiofauna, by no means less well represented in the deep sea than in the shallows.

In the remaining two-thirds of the book we move away from descriptive zoology to present-day quantitative ecology. Part three, 'Patterns in Space' describes the small-scale distribution of the fauna, random or not, its abundance and the existence of gradients.

There follows a discussion of diversity in the deep sea, a topic of active controversy. The authors stress that rarefaction curves for most taxa indicate that new species will still be found as sampling increases. They also note that hypotheses about equilibrium states require acceptance of slow rates of colonization of the deep sea. From distribution patterns and diversity they move on to the vexed question of depth-related faunal zones, as yet difficult to answer, though it is noted that in general the environment tends to become more uniform with greater depth. In the chapter on speciation and the origin of the deep-sea fauna, the authors find that hard data are scarce. But they note that the lower densities of individuals in the deep sea could lead to greater divergence and genetic drift. There is a long review of the problems of determining the age of deep-sea communities, but this fails to note examples of archaic relicts described from new hydrothermal vents, notably the stalked cirripedes.

Part four, 'Process and Patterns in Time', opens with a description of food sources, including detritus from the land, which can be important in trenches and basins close to land masses. The concept of dead whales and large fish reaching the sea bed and possibly supporting scavengers with enhanced chemoreception facilities is also reviewed, but predominance is given to fast sinking of finer particles. The fascinating work of the past ten years is described, showing how photography and biochemical assay prove that the remains of plankton

blooms at the surface can reach the deep-sea bed within a month. This introduces a strong seasonal signal in growth and reproduction in what was once thought to be a more-or-less unchanging environment.

After reviewing what is known about deep-sea metabolic patterns, including bacterial life, the authors discuss the variety of reproductive strategies found in the deep sea. It may come as a surprise to learn that some species living at great depths can produce planktonic larvae that live for a while in the

energy removes dependence of the fauna on euphotic-zone production, is still the fastest advancing topic of deep-sea researches, but in spite of the volume of work produced so far much more study is required to understand the ecology and spread of vent faunas. Thus there is little evidence that the majority of vent species have lecithotrophic larvae, and indeed some of the references quoted describe planktotrophic larvae emitted in the large numbers that may be necessary to ensure survival of animals inhabiting short-lived vents. The authors also

appear confused over carbon sources, particularly the role of carbon isotope ratios, which are primarily of use in following trophic pathways rather than in assessing age of the carbon.

Chapter 16 is recommended reading for politicians as it deals with exploitation of the deep sea through fishing, mineral extraction and waste disposal, most in the form of proposals rather than current active involvement. So far the deep sea has been protected by technological problems, but recent exploitation of artefacts from deep shipwrecks underlines the need for caution. Much research into deep-sea ecology has been funded by governments and organizations requiring a place to dump

radioactive and other toxic wastes, so the results appear in 'grey' literature or unpublished symposium contributions. The authors give some prominence to a smug report by the UK Nuclear Energy Authority, but other studies, not mentioned, suggest that drums used to encase low-level waste dumped in the deep sea have a short life. Some mention might also have been made of the pumping of pharmaceutical waste of high biological activity into the Puerto Rico trench, and related changes in the bacterial flora.

This is a landmark study, a well-documented and lavishly illustrated account of almost all aspects of life at the bottom of the deep sea. There are some typographical errors in addition to the few textual misunderstandings that need clearing up for the next printing, but the book is strongly recommended as essential reading for all courses in marine biology. Hopefully, the publisher will issue an affordable edition for students. □

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Deep-sea grazers — a 'herd' of the elaspodid holothurian *Scotoplanes globosa* on the floor of the San Diego Trough at a depth of about 1.2 km.

surface waters. It is a short step from breeding to describing growth rates, mostly from circumstantial evidence. By reading rings on shells and other hard skeletal components we find a range of growth rates, some of which, the authors report, are not so dissimilar, after all, from rates of corresponding shallow-water representatives. Turnover rates of deep-sea populations may also compare with those of shallow-water communities. The last chapter in this section deals with the tantalizing topic of animal-sediment relationships, so much based on conjectural analysis of photographs and sections of box cores. Many researchers would love to know just which animals leave the traces on the mud surface, dig the burrows, and pile up the mounds visible in bottom photographs, but the authors can only whet our appetite and leave us to await more studies.

The final section, 'Parallel Systems and Anthropogenic Effects', includes a review of hydrothermal vents and cold seeps as well as an assessment of waste disposal in the deep sea. The specialized faunas that occur around heated vents and cold seeps, where geochemical

On best behaviour

Mark Ridley

Man and Beast Revisited. Edited by Michael H. Robinson and Lionel Tiger. Smithsonian Institution Press: 1991. Pp.386. \$16.95, £13.25 (pbk).

ANIMALS, whether human or non-human, often have to sort out conflicts of interest; and as they more-or-less metaphorically size each other up to plan their next move, there may well be an advantage in deceiving each other about their strength, social connections, sex appeal, and so on. Deception only works if it is not seen through, and natural selection should act in these cases to make it as effective as possible. One effective means (as several biologists have argued) is for the deceiver first to deceive itself. We lie most convincingly when we believe we are being honest. Robert Trivers — who has done as much as anyone to inspire this line of thought — develops the idea in a marvellous essay in this multi-authored conference volume, a successor to a 1969 Smithsonian conference called *Man and Beast*.

Trivers argues that an animal will often know how angry, powerful, or whatever, it really is, but will repress that knowledge into its unconscious while holding in its consciousness ideas better suited to social advancement. "True and false information are simultaneously stored in the same individual; the true information is in the unconscious, the false information in the conscious." He describes illustrative experiments, using the galvanic skin response, for humans and suggests how analogous work could be done with nonhumans. If Trivers is right, we should be particularly likely to hold contradictory conscious and unconscious models of reality for information that matters in conflicts of interests and is capable of being communicated. In his words, his aim is to explain something that "everyone seems agreed" about: that "there is some kind of intimate connection between communication and consciousness".

Trivers takes seriously the possibility that many species (perhaps including plants) are conscious, and he describes his attempts to involve the neighbourhood birds in conversation. He has been blowing through an old whistle at the local "mockingbirds at 2 o'clock in the morning outside [his] home in Santa Cruz". "Whether they make any more sense of this than I, I cannot tell", he concludes, though he has enjoyed some extended bouts of countersinging with them. He recommends the experiments



Wooden waterwheels, such as these, kept the gardens of Cairo in bloom between the tenth and twelfth centuries, when the city was the centre of the Islamic world. *A Forest Journey* by John Perlin, which traces the role of wood in the development of civilization, is now published in paperback by Harvard University Press at \$14.95, £11.95. □

not only on "scientific grounds" but also for "raising one's own consciousness": and the scientific community will have to decide whether Trivers's ideas are more conveniently (and quite nonjudgmentally) termed the 'Californian' or the 'Franciscan hypothesis'.

The chapters are not all as enjoyable as Trivers', but he is in many ways representative. The book's main (but certainly not universal) theme is behavioural ecology; all the chapters should be intelligible to the non-specialist; many are highly personal in style (Michael Robinson's, in the extreme, is pure autobiography); most are resolutely nonempirical. Thus J. H. Crook, who has been not merely to California but up the Himalayas, provides a chapter on consciousness that is even more 'spaced-out' than Trivers'; and Richard Dawkins concludes an elegant chapter (which includes, by the way, good material on evolution and the second law of thermodynamics) by inviting "empirically minded, white-coated, gum-booted biologists" all to equip themselves with armchairs. They should then be able to draw rather less parochial conclusions — conclusions indeed that "do not require us actually to know any facts at all".

On humans, Helen Fisher's chapter reviews the evidence on divorce. She has compiled data from a number of societies, and together they suggest that the frequency of divorce peaks about four years after marriage. She connects this with the four-year interbirth interval known from several hunter-gatherer societies, and tentatively conjectures that human monogamy may be serial: couples may stay together to produce one offspring and then change partners. The argument, as presented, has too many gaps to convince, and the analysis is incomplete; but it may become an

important study. In his chapter, Richard Potts synoptically outlines modern work on the time at which the uniquely hominid characteristics evolved, and includes his own views on hunter-gatherers: he believes the hunter-gatherer phase of our evolution originated only about 700,000 years ago, rather than over two million years ago.

Even by the standards of the genre, *Man and Beast Revisited* is a mixed bag. It contains 21 chapters; but this number includes some remarkably slight offerings by senior scientists — as if the book is intended to commemorate the symposium rather than contribute to the literature. The other longer chapters include three on pets, one (by Phyllis Dolhinow) on the promising topic of 'tactics of primate immaturity' (that is, how to behave if you are a young monkey), and a chapter by Restak, who lists several (unconvincing to me) differences between brains and computers. If that is not variety enough, the mainly biological core is sandwiched between a final chapter by the climatologist Stephen Schneider on global warming and an opening chapter, by the astrophysicist Irwin Shapiro, on the origin of the Universe. There is also a dyspeptic preface (or "personal note") by Thomas Sebeck, which is out of character with the rest of the book. "It should be noted", he notes, "that many animal behaviourists continue to habitually confound three radically different concepts: communication, language, and speech." Well, we must know different animal behaviourists. *Man and Beast Revisited* is one of those books that make us grateful for the photocopying machine and the nonenforcement of the copyright laws. □

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Blue stragglers in the core of the globular cluster 47 Tucanae

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High-resolution observations of the core of the globular cluster 47 Tucanae with the Faint Object Camera on the Hubble Space Telescope reveal a high density of 'blue straggler' stars, occupying the upper end of the main sequence from which all stars in the cluster should have long since evolved. Their presence in the dense core supports the hypothesis that they formed by stellar collision and coalescence, and, as the heaviest objects in the cluster, have drifted to the core.

STELLAR densities in the cores of globular clusters ($\sim 1000 M_{\odot} \text{pc}^{-3}$) are thousands of times higher than in the solar neighbourhood ($\sim 0.065 M_{\odot} \text{pc}^{-3}$). Close encounters with tidal interactions¹ and physical collisions^{2,3} between stars should be commonplace in the densest parts of all but the sparsest globular clusters. The products of such events may include cataclysmic and low-mass X-ray binaries¹, massive disk stars^{4,5} and blue stragglers^{3,6}. The very high space densities which make globular cluster cores so interesting to theorists are the bane of observers searching for the remnants of close encounters or collisions. The central regions of a few globular clusters have recently been shown to host blue stragglers⁷⁻⁹ which are more centrally concentrated than the cluster subgiants at the same magnitude. High-density clusters are generally difficult to resolve from the ground, and have not yet been searched to magnitude limits faint enough to detect blue stragglers.

These stars correspond to a sparse upward extension of the main sequence (MS) above the cluster turn-off and are situated on a locus of the colour-magnitude diagram (CMD) previously occupied by stars that have now evolved away from the MS. First noticed by Sandage¹⁰ in M3, they have now been observed in other globulars, in both old and young open clusters and even among field halo stars¹¹. The three most probable ways in which they could be formed are coalescence after direct stellar collision, an extended MS lifetime because of mass transfer in binaries or an extended MS lifetime because of internal mixing in single stars.

The high spatial resolution and ultraviolet sensitivity of the Hubble space telescope (HST) make it a potentially powerful tool for probing the dense centres of globular clusters. Despite the spherical aberration of the HST optics, ~ 0.1 arcsec resolution is routinely obtainable for moderately bright point sources. During the science verification phase of HST, we imaged the

high-density core of the globular cluster 47 Tuc both to check the focus variations of the Faint Object Camera (FOC) and to investigate the nature of the stars in the cluster's core. The results of the latter study are reported here.

Observations

The data presented here were obtained on 16 November 1990 with the FOC in its F/96 mode and on 28 November 1990 in the F/48 mode. The FOC is described in detail elsewhere^{12,13}. A series of eight exposures, each of duration ~ 10 min, were taken with the F140W and F220W F/48 filters, and four exposures of the same duration were taken with the F130M and F220W F/96 filters, while the FOC internal refocus mechanism was stepped through its nominal 16-mm-wide range for calibration purposes. The telescope was pointed at the nominal optical centre of 47 Tuc determined by Grindlay *et al.*¹⁴. The images taken around the internal best-focus position of the F/96 camera yield a point spread function of ~ 70 milliarcseconds (mas) FWHM, whereas the corresponding F/48 images have a 160-mas FWHM. The encircled energy within a 100-mas radius of the central peak corresponds to 15% of the total energy for the F/96 images and 9.5% of the total energy for the F/48 images at these wavelengths. The rest of the light is spread out over a circular halo of ~ 2 arcsec radius because of the spherical aberration of the primary HST mirror¹³.

The F130M and F220W F/96 filters have peak transmission at 1,280 and 2,260 Å respectively, and the corresponding values for the F140W and F220W F/48 filters are 1,320 and 2,250 Å. Because of the extended red wings of the ultraviolet wide-band-filter transmission curves, however, the mean wavelengths for the F140W and F220W filters are 1,645 and 2,300 Å, respectively. The complete F/96 system was calibrated absolutely using the standard star BPM16274 (ref. 15). The overall efficiency at the wavelengths of interest was 75% of the nominal system response tabulated in the FOC instrument handbook¹². The F/48 relay was calibrated by direct comparison of the same stars covered by the F/96 relay. The raw images were flattened and geometrically corrected to remove the optical and detector distortions. To maximize the signal-to-noise ratio of faint stars, several of the best frames in the series were then registered and added. The final F/96-F220W image used here thus corresponds to an effective exposure time of 38.7 min, the final F/48-F220W image to 27.8 min and the final F/48-F140W image to 55.3 min.

The results of this processing are shown in Figs 1 and 2. The F/48-F220W field centred on the 47 Tuc core is shown in Fig. 1, and the higher resolution F/96-F220W image of the central portion of this field is shown in Fig. 2. The slight rotation

between the two images is due to the differing camera positions and roll angles of the spacecraft during the sets of data. The orientations on the sky are indicated at the lower corners of the images. Both images are negatives and the grey scale is linear from 0 to 250 counts per pixel. No pixel in the frame exceeds the limiting count rate for strictly linear response. Assuming 47 Tuc is at a distance of 4.8 kpc (ref. 16), 1" in these images corresponds to a linear scale at the source of 0.023 parsecs. Three available determinations of the centre of the cluster are marked with the letters A (ref. 17), M (ref. 18) and G (ref. 14).

Photometric results

About 600 stars are discernible in the F/48-F220W frame and about 300 in the F/96-F220W and F/48-F140W frames. All the F/96-F130M images are completely empty, a fact which yields additional astrophysical constraints (see below). The overlap of the extended haloes around the brighter stars, caused by aberrations in the HST point spread function, gives rise to the faint nebulosities apparent in the regions surrounding these stars. This effect, of course, not only decreases the observable limiting brightness but also reduces the accuracy of the achievable photometry in those areas. As the ultraviolet images shown in Figs 1 and 2 are only moderately crowded, however, good results can be obtained by calculating the stellar flux in a very small aperture, of diameter roughly equal to the FWHM of the sharp core of the point spread function, and the background in an annulus centred on the sharp central peak but contained well within the area of this function. Flux normalization is provided by the known overall response and the encircled energy curves of the F/96 relay to which the F/48 relay can be referred in the overlap regions at the centre of the frames.

Standard photometry routines were used to calculate the fluxes of stars in the F/48-F140W and F220W frames. These routines located ~ 125 stars with well defined fluxes in both colours corresponding to a threshold of 5σ above the frame-averaged background. The results are shown in Fig. 3 in the form of an ultraviolet CMD in which the magnitude in the F220W frame, m_{220} , is plotted against $m_{140} - m_{220}$, which is appropriate to the inner $20 \times 20 = 400$ arcsec² region of 47 Tuc.

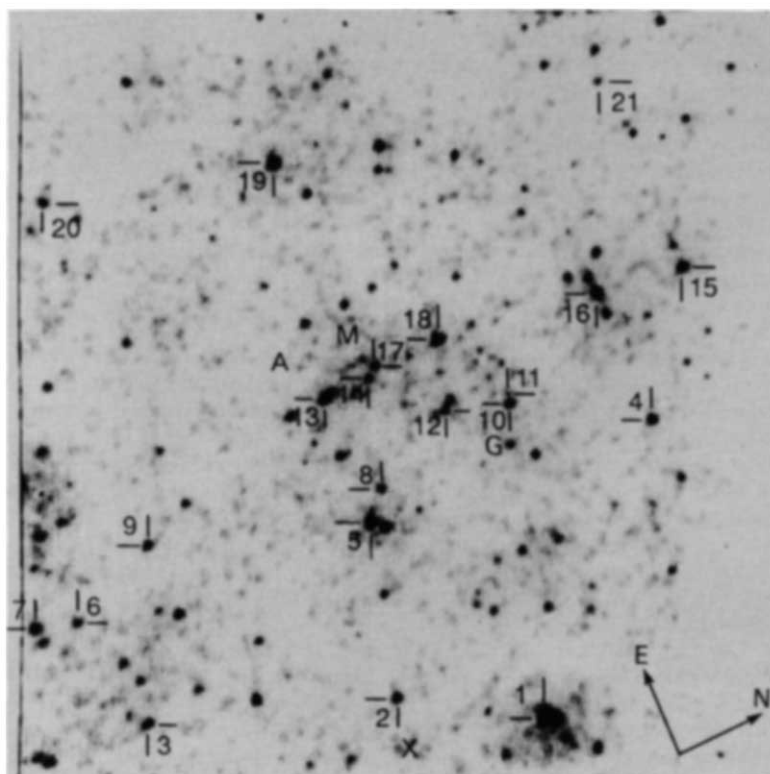
TABLE 1 Positions and magnitudes of the blue stragglers in 47 Tucanae

Name	x_{220}	y_{220}	m_{220}	m_{140}	B	V
HST-1	157	47	16.0	16.7		
HST-2	252	62	17.6	18.3	16.8	16.0
HST-3	411	46	18.0	18.5		
HST-4	86	241	17.3	18.1	16.7	16.4
HST-5	268	175	16.8	17.5		
HST-6	456	111	18.0	19.0		
HST-7	483	108	17.3	18.2		
HST-8	261	197	17.7	18.4	16.5	
HST-9	411	162	17.7	17.8	15.9	15.0
HST-10	178	252	17.6	18.3	16.0	
HST-11	179	258	18.8	19.1		
HST-12	219	248	18.2	18.5		
HST-13	297	255	16.9	17.5		
HST-14	269	269	18.1	18.7		
HST-15	65	342	17.3	18.1		
HST-16	120	324	17.3	18.0		
HST-17	266	277	18.0	18.4		
HST-18	225	294	16.9	17.6	16.3	16.2
HST-19	330	409	16.3	17.1	16.1	16.2
HST-20	478	384	17.9	18.5	16.6	16.6
HST-21	120	463	18.3	19.0		

The HST instrumental magnitudes are defined as $m = -21.1 - (2.5 \times \log(Uc))$, where U is the inverse sensitivity of the instrument mode used in $\text{erg cm}^{-2} \text{\AA}^{-1}$, and c is the calculated total signal count rate attributable to the star¹⁹. In our case, $U = 8.83 \times 10^{-17}$ and 1.74×10^{-17} for the F/48-F140W and F220W modes, respectively. With the quoted exposure times and efficiencies, the 5σ threshold applied to the data corresponds to a limiting m_{220} magnitude of ~ 20 . From all the uncertainties involved, we estimate a 1σ error of ± 0.1 magnitude in m_{220} and m_{140} for stars brighter than 19 growing to a few tenths of a magnitude for fainter stars.

The content of our unusual ultraviolet CMD can be understood by mapping the visual (V against $B - V$) CMD into the ultraviolet CMD with the help of its corresponding visual CMD

FIG. 1 F/48-F220W image of the core of 47 Tuc. The field is 22×22 arcsec² in size and is oriented as indicated by the arrows. A, M and G indicate the position of the cluster centre as determined by refs 17, 18 and 14. X is approximate centre of the X-ray source error box as given by ref. 22. Numbered objects are blue stragglers whose properties are listed in Table 1.



determined from ground-based observations. This is done by running the known spectra of stars of the appropriate luminosity class and spectral type obtained from the Bruzual–Persson–Gunn–Stryker (BPGS) library of stellar spectra (G. Bruzual and E. Persson, personal communication) through the FOC instrument simulation software FOCSIM. The latter is set to the absolute calibration described above and the filter transmissions as a function of wavelength measured on the ground before flight. In general, the redder the star, the more negative the corresponding $m_{140} - m_{220}$ colour, owing to the slightly higher transmissivity of the F140W filter beyond $\sim 5,000 \text{ \AA}$.

The two curves to the right of the $m_{140} - m_{220}$ zero point (the origin) in Fig. 3 represent the loci of the expected positions of stars on the mean composite visual CMD of Hesser *et al.*¹⁶. Specifically, the two lines trace the locations in the ultraviolet CMD of the mean red-giant branch down to the main sequence, and the mean asymptotic giant to the red horizontal branch, as defined in Table IX of Hesser *et al.*¹⁶. In implementing this procedure, we have made the implicit assumption that the positions of the main sequence and these three branches are similar for fields in the core and in the outer regions of the cluster, as confirmed by Aurière and Ortolani¹⁷. These stars, in essence, are all those brighter than $V = 17$ (roughly down to the turn off).

Most of the stars of the sample shown in Fig. 3 lie to the right of the origin and cluster around the mean positions of the known structures of 47 Tuc's CMD. By contrast, the bright stars to the left of the origin constitute a well defined population of ~ 20 stars brighter than $m_{220} = 19$ with a definite positive $m_{140} - m_{220}$ colour. Their red colour here simply reflects the fact that they are significantly fainter below $2,000 \text{ \AA}$ than above. They are labelled as HST-*n* sources in Table 1 and identified by their numbers in Figs 1–3. They cannot be attributed to any known stellar population in the Hesser *et al.*¹⁶ visual CMD appropriate to their F1 and F2 fields.

This conclusion depends on the adequacy of the CMD obtained by Hesser *et al.*¹⁶ in the core down to the turn off and on the correctness of the visual-to-ultraviolet CMD mapping parameters used in our investigation. To analyse these issues in more detail, we compared our HST images with two short

exposure B and V images of 47 Tuc, taken in 1" seeing by one of us on 12 December 1988 with the ESO/MPI 2.2-m telescope of the European Southern Observatory at La Silla, Chile. The limiting magnitude of these frames is $V \approx 16.5$. This ground-based data was used to derive the visual CMD in the core to this limiting magnitude. The photometry of a part of this field published by Aurière *et al.*²⁰ was used to set the magnitude scale. The stars clearly detectable in the $22 \times 22 \text{ arcsec}^2$ field that was covered by the F/48 images (roughly 100 stars) reproduce fairly accurately the expected features of the Hesser *et al.*¹⁶ CMD determined much further away from the core in their fields F1 and F2. This result confirms that, for stars brighter than $V = 16$, there are no significant deviations of the upper part of the visual CMD in our small field in the core of the cluster. It also allows us to confirm the correctness of the pre-launch instrument parameters required to map the visual CMD into the ultraviolet CMD shown in Fig. 3, by establishing the direct correspondence between the two diagrams object by object. The sum of the observed and calculated fluxes in the F220W bandpass of the stars in the F/48 field (Fig. 1) yields an integrated flux of $\sim 2 \times 10^{-14} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ \AA}^{-1}$, a value consistent with the spectrum of the 47 Tuc core obtained by the International Ultraviolet Explorer²¹.

Blue stragglers in the core

During the verification, we discovered that hardly any of the HST-*n* stars had appreciable flux in the B and V frames, confirming their exceptional nature. On closer inspection of the B and V frames, however, a few are detectable, more reliably in the B and marginally in the V band. In Table 1, we list the positions and apparent brightness of the 21 ultraviolet-bright stars we have discovered in our observations. The positions are given in the *x, y* coordinates corresponding to positions in the F/48–F220W image shown in Fig. 1, with the origin at the bottom right corner. They are determined as the centroid of the best gaussian fit to the source–signal distribution. The ultraviolet magnitudes are HST magnitudes and the B, V values are the standard Johnson colours. Except for HST-9, all the visual fluxes are at or near the detectability limit of the visual frames and

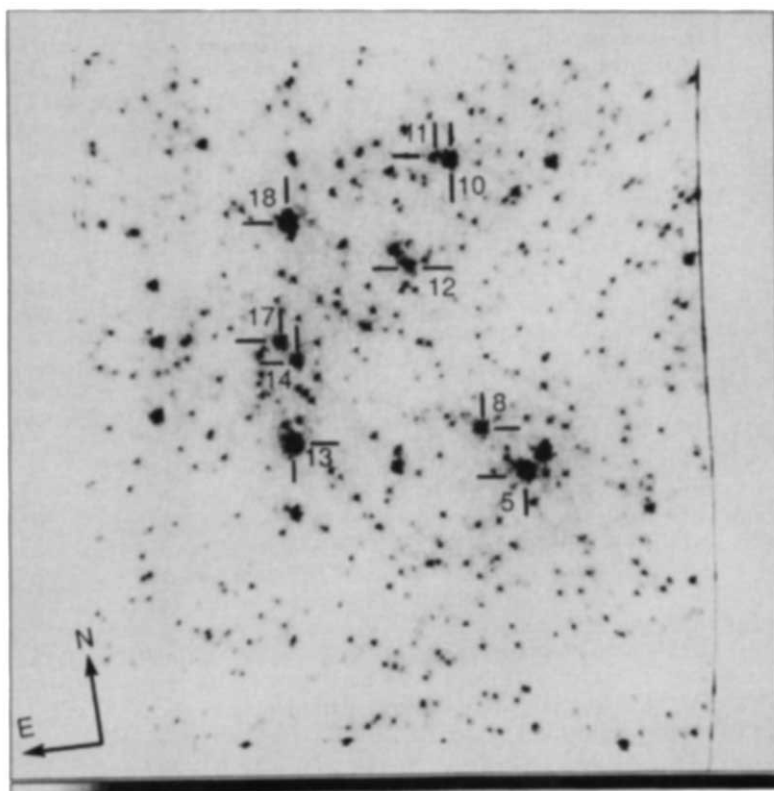
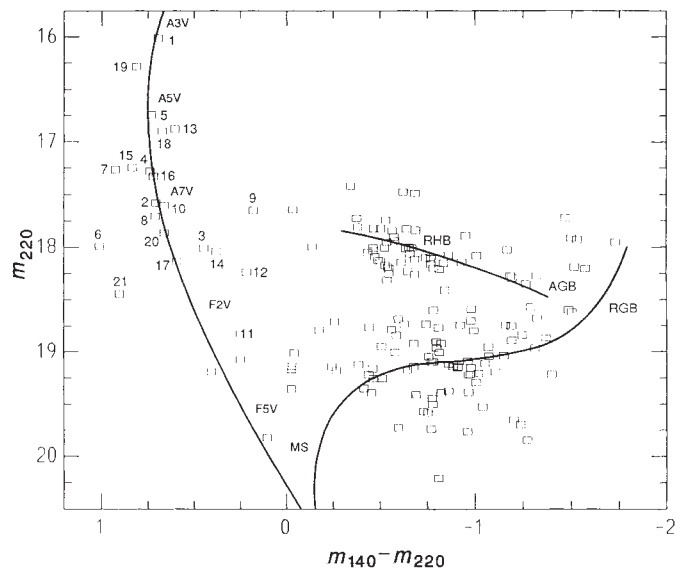


FIG. 2 F/96–F220W image of the core of 47 Tuc. The field is $11 \times 11 \text{ arcsec}^2$ and corresponds to the inner portion of the field shown in Fig. 1.

FIG. 3 The ultraviolet colour-magnitude diagram of the stars (squares) detected in both the F/48-F220W and F/48-F140W fields. The m_{140} and m_{220} are HST instrumental magnitudes defined in the text. Solid lines to the right of the origin, expected locations of the red giant (RGB), asymptotic giant (AGB), red horizontal (RHB) and main-sequence branches in the mean composite visual colour-magnitude diagram of Hesser *et al.*¹⁶. Solid line to the left of the origin, upward extension of their main sequence with the approximate positions of the corresponding spectral types indicated. Blue stragglers marked with the numbers given in Table 1.



have, therefore, an associated error of $\sim \pm 0.5m$. The B fluxes are more firmly established in most cases and are probably correct to $\pm 0.2-0.3m$. The other stars with no visual data listed are those for which their inherent faintness in the visual frames and/or their location within the seeing-dominated point spread function of nearby bright giants precludes any meaningful measurement. Judging from their ultraviolet colours, however, we would expect most of these also to lie near or just below the $V = 16.5$ threshold.

The visible flux from one source (HST-2) was measured by Aurière *et al.*²⁰, who find $U = 16.73$, $B = 16.83$ and $V = 16.01$, and, therefore, $B - V = 0.82$ and $U - B = -0.10$ for this star (numbered 6 in their Table 6). This star is located inside the error circle of the position of the X-ray source contained in the core of 47 Tuc. An X marks the approximate centre of this circle in Fig. 1. Aurière *et al.*²⁰ consider their star number 9 as the best candidate for the visual counterpart of the X-ray source, but the star is barely detectable in our observations and is not contained in the much smaller error box published by Bailyn *et al.*²². HST-2 remains the only object in the latter error box, with by far the bluest $U - B$ index.

The position in the ultraviolet CMD of the stars listed in Table 1 and the absence of any detectable object in the F/96-F130M frames suggest that they are objects with effective temperature $T_e \leq 10^4$ K but above the temperature corresponding to the MS turn off. To confirm this, a series of stellar models from the BPGS library corresponding to the expected position of an unevolved MS whose lower part matches the one observed by Hesser *et al.*¹⁶ were run through FOCSIM. The results are shown in Fig. 3 in the form of the solid line to the left of the origin, spanning the range of MS spectral types from $\sim$ F5V to A3V as indicated. The excellent match allows us to conclude that most of the HST stars detected are blue stragglers. Stars of this spectral type and luminosity are not detectable in the F/96-F130M band, both because they are too cool and because the sensitivity of this mode is about one-tenth of the F/96-F140W mode¹². The possibility that a considerable fraction of these objects actually belong to the background Small Magellanic Cloud can be easily disposed of by noting their inherent brightnesses and their complete absence from the sample in the F1 and F2 fields analysed by Hesser *et al.*¹⁶. The high galactic latitude of 47 Tuc also considerably reduces the chance that they are foreground field stars.

The observations reported here and model calculations strongly imply that the HST stars all have $16 < V < 18$ and $0.1 < B - V < 0.5$, approximately. A very small number of such objects do appear in the F3 and F4 fields of Hesser *et al.*¹⁶. Although they would be situated at or just below Aurière and Ortolani's¹⁷ detectability limit, a few may be present in that data

set as well. What is certainly clear is that the high surface density of the blue stragglers we have discovered in the core (1 star per 20 arcsec²) is not maintained out to the periphery. The surprising result of a very centrally peaked space density of blue stragglers explains the fact they had up to now eluded detection with instruments that do not have the required high spatial resolution to resolve the dense core of 47 Tuc.

This gradient in the density of blue stragglers towards the core of 47 Tuc further supports the hypothesis that blue stragglers are significantly more massive than cluster main sequence and subgiant stars, and could constitute a population of binaries²⁵. Because of the short relaxation time in the core of 47 Tuc, $\sim 10^7$ yr (ref. 24), which is much less than the cluster age of ~ 15 Gyr (refs 16, 25), the inner parts have been driven by two-body relaxation into a state characterized by isotropy of the velocity dispersion. In this relaxed core, mass segregation should have taken place through equipartition of energy, the most massive stars sinking towards the inner parts. Our observations, taken together with those of Hesser *et al.*¹⁶, indicate a gradient in the population of blue stragglers, supporting the suggestion that blue stragglers are probably binaries, and thus amongst the most massive stars in the cluster. These binaries contain enough energy to halt core collapse and even drive re-expansion of the inner part of the cluster.

We are unable to distinguish observationally between the three possibilities for the nature of the blue stragglers of 47 Tuc listed earlier without additional data on time variability and spectra. The presence of this population of possible binaries is interesting from the point of view of the high-velocity stars found in the core of 47 Tuc by Meylan *et al.*¹⁸, which could only be the result of gravitational interactions between single stars and binaries, or between two binaries. The recent discovery of a considerable population of millisecond and binary pulsars in 47 Tuc²⁶ also adds weight to the conjecture that binaries are present in this cluster. $\square$

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Sub-piconewton force fluctuations of actomyosin *in vitro*

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A new system has been developed for measuring the forces produced by a small number (<5–150) of myosin molecules interacting with a single actin filament *in vitro*. The technique can resolve forces of less than a piconewton and has a time resolution in the submillisecond range. It can thus detect fluctuations of force caused by individual molecular interactions. From analysis of these force fluctuations, the coupling between the enzymatic ATPase activity of actomyosin and the resulting mechanical impulses can be elucidated.

MUSCLE contracts by the relative sliding of actin and myosin filaments fuelled by the chemical energy from hydrolysis of ATP (the sliding filament theory)^{1,2}. But in spite of much investigation the elementary processes in this chemo-mechanical energy transduction have remained obscure. This problem has probably been difficult to solve because the muscle fibres and suspensions of purified proteins generally used are too complex to provide unambiguous information. To alleviate this, it is essential to develop new techniques that probe the mechanical processes directly at the molecular level.

Much attention has been focused on *in vitro* motility assays. The myosin-coated surface assay allows us to observe motions of single actin filaments produced by isolated myosin molecules or subfragments bound to a substrate³. Development of this *in vitro* motility assay followed the design of techniques for direct observation of single actin filaments labelled with fluorescent phalloidin using optical microscopy⁴. It has been used widely and has provided insights into the relationship between the structure and function of myosin^{5–9} and the chemo-mechanical coupling in energy transduction^{10–12}. Furthermore, it is now possible to measure force *in vitro* by manipulating single actin filaments attached to glass microneedles¹³.

Here we describe extensions to the myosin-coated surface assay that allow measurement of the force produced by a small number of myosin molecules. The resolving power of the technique has been enhanced to the sub-piconewton and sub-millisecond ranges. Many of the mechanical experiments that were done using muscle fibres are possible with this method, but also it makes possible the resolution of fluctuations in force caused by individual molecular interactions.

Analysis of force fluctuations (noise) is a very powerful

approach for determining kinetic characteristics of individual myosin heads interacting with actin^{14,15}, especially when the number of interactions is small. Large force fluctuations observed under nearly isometric conditions (with minimal sliding of the filaments) are similar to membrane current fluctuations due to channel gating in electrophysiological systems with small numbers of channels¹⁶.

The force fluctuations caused by mechanical interactions between myosin heads and actin in the isometric condition are consistent with models incorporating stochastic and independent molecular events. Both a simplified, two-state, 'on-off' model and the Huxley model¹⁷ simulate the amplitude and frequency spectrum of the observed noise. Using these models we determined the on and off rates and the force generated by single myosin heads. The data are compatible with one fundamental mechanical interaction for each ATPase cycle (ATP hydrolysis cycle).

When the actin filaments actively slide at velocities greater than about $1 \mu\text{m s}^{-1}$, fluctuations of force are much smaller. The data analysis indicates that during an ATPase cycle, myosin produces a nearly constant force for most (probably 90%) of the ATPase cycle time. This suggests that during sliding, for each ATPase cycle there are multiple force-generating mechanical interactions between actin and myosin.

Thus, the present system allows us to examine directly how the ATPase cycle is coupled to the mechanical interactions of individual myosin heads with actin *in vitro*. These conclusions also apply directly to the molecular mechanism of muscle contraction.

Ultra-high-resolution force measurements

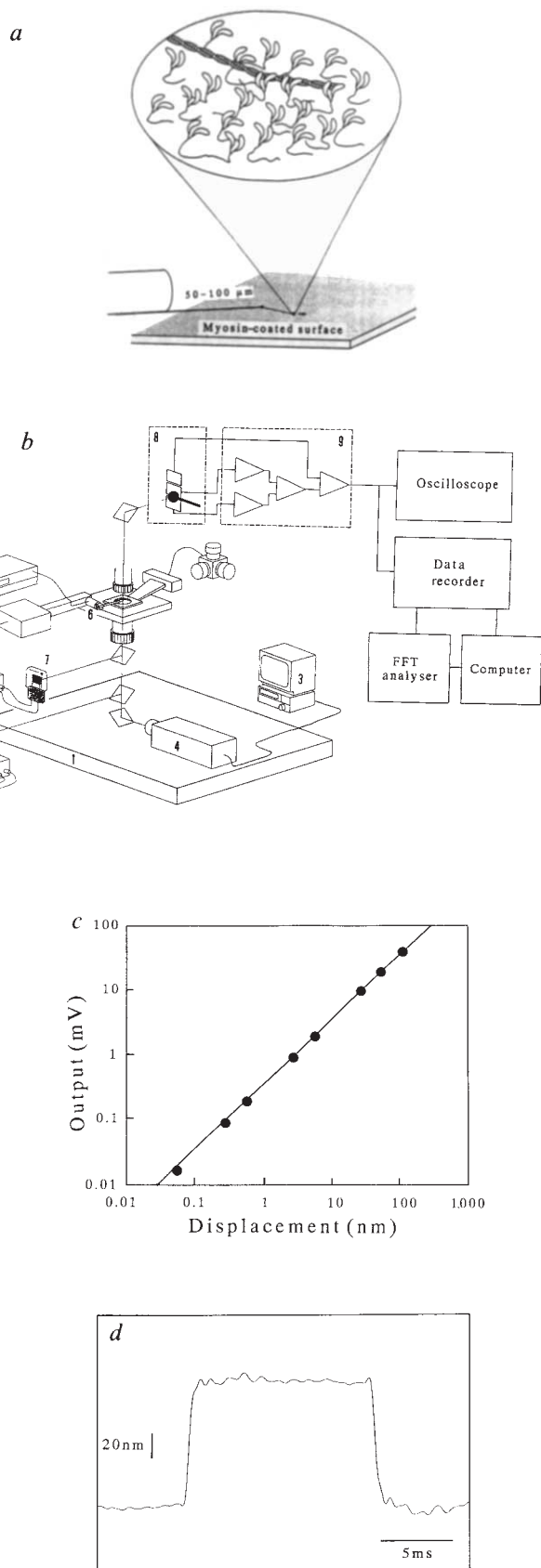
Single actin filaments were labelled with rhodamine phalloidin, attached to glass microneedles and manipulated in an inverted fluorescence microscope essentially as previously described¹³. One end of a 10–20- μm -long actin filament in solution was caught by *N*-ethylmaleimide (NEM)-treated myosin on a microneedle. The other end of the filament was brought into contact with a myosin-coated coverslip (Fig. 1a). In the presence of ATP, actomyosin interactions caused sliding of the filament and bending of the needle. On the basis of the measured stiffness of the microneedle, the bending could be interpreted quantitatively as force generation by actomyosin. The number of myosin molecules interacting with the thin filament could be varied by adjusting the length of filament in contact with the surface. The amount and the velocity of sliding depended on the length of filament interacting with the surface divided by the needle stiffness. If this ratio was small, the total sliding was <50 nm. This condition was considered nearly isometric. If a longer length of filament interacted or the needle was less stiff, the

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FIG. 1 *a*, Micromanipulation of a single actin filament by a glass microneedle. The angle between the axis of the actin filament and the myosin-coated surface was set to about 20°. The stiffness of the needle (K_n) was determined in two ways. In the first method, needle stiffness was cross-calibrated against a reference needle of known stiffness as described previously¹³. In the second method, K_n was obtained from mean squared thermal vibration ($\langle \delta X_n^2 \rangle$) using the relation $\frac{1}{2} K_n \langle \delta X_n^2 \rangle = \frac{1}{2} k_B T$, where k_B is Boltzmann's constant⁴⁸. The stiffness values obtained by the two methods were similar. *b*, Schematic diagram of the ultra-high-resolution force measurement system.

The setup was built on an inverted fluorescence microscope (Olympus, IMT-2), modified to minimize mechanical vibrations and drift, and set on a vibration-free table (1, Heltz, HG-107LM). Fluorescence of the labelled actin filament was excited by 540 nm light from an ultra-high-pressure mercury lamp (2) and a $\times 100$ oil immersion objective lens (Zeiss Neofluor, NA 1.3). The fluorescent image of the filament was displayed on a TV monitor (3) by a high-sensitivity SIT camera (Hamamatsu-Photronics, C 2741) (4). The microneedle catching the actin filament was manipulated by a fine micro-manipulator (Narishige, WR-88) (5) and a piezo-actuator (CTC, CTC-6094-5) (6). The displacement of the needle was measured as follows. The nickel particle was illuminated with 650–900 nm light from a highly stabilized halogen lamp (7) with $<0.2\%$ ripple of light intensity. The image of the nickel particle was projected onto a pair of photodiodes (Hamamatsu-Photronics, S2545) (8) through an objective lens (Olympus, ULWD CDPlan $\times 40$) with a long working distance of 5 mm. The photodiode outputs, converted into voltage by I - V converters, were subtracted, referred to the incident light intensity by a divider circuit (Analog devices, AD-515) (9), and recorded on tape by a digital data recorder (TEAC, RD-1010T). The differential signal was analysed off-line by a fast Fourier transform (FFT) analyser (Advantest, R9211A) and a computer (NEC, PC-9801). *c*, Changes in the differential output when sinusoidal displacements were applied to the needle with amplitudes of 0.06–100 nm and a frequency of 100 Hz. The output amplitudes were obtained from the power spectra using the FFT analyser. *d*, Time course of the movement of the needle when sudden length changes of 100 nm were applied to the base of the needle by the piezo actuator. The trace was obtained by averaging five scans. The rise time (0–63%) was 0.4 ms. This trace was obtained using the most flexible needle (0.09 pN nm^{-1}) used in this work. The ratio of inertia to viscous forces for the needle, for example when a 20 nm, 1 kHz sinusoidal motion is applied to it, is estimated to be $<10^{-2}$. Therefore, for interpretation of the experimental data, the inertia of the needle with the nickel particle can be neglected. In this case, the equation of motion for the needle gives the rise time as $\tau = \xi_n / K_n$, where ξ_n is the coefficient of viscous drag and K_n is the stiffness. Thus, $\xi_n = \tau K_n = 0.036 \text{ pN ms nm}^{-1}$, which agrees well with the theoretical value⁴⁹. In the experimental situation, the rise time τ' of the needle's motion driven by actomyosin interaction is similarly given by ξ_n / K_{comp} , where K_{comp} is the composite stiffness of the needle, actin filament, actomyosin attachments and the bonds between the actin filament and the needle, which are all connected in series. The viscous drag between the solution and actin and myosin is negligible compared with ξ_n and for the various needles used, ξ_n is considered to be about the same. Because the stiffness of an actin filament (A. Sano and T.Y., personal communication) and the needle-filament bond are both $>5 \text{ pN nm}^{-1}$, K_{comp} is determined by the stiffness of the actomyosin attachments, K_{mN} , and the stiffness of the needle, $K_{\text{comp}} = K_{\text{mN}} K_n / (K_{\text{mN}} + K_n)$. Under isometric conditions (Fig. 3), the stiffness of a single head attached to actin is 0.018 pN nm^{-1} . Considering a mechanical impulse produced by only a single head that is measured using a needle with stiffness of $K_n = 0.09 \text{ pN nm}^{-1}$, $K_{\text{comp}} = 0.015 \text{ pN nm}^{-1}$ and τ' would be $\xi_n / K_{\text{comp}} = 2.4 \text{ ms}$. In practice, more than one myosin head interacted with the filament and needles with stiffness $\geq 0.09 \text{ pN nm}^{-1}$ were used, so $K_{\text{comp}} > 0.03 \text{ pN nm}^{-1}$ and $\tau' < 1 \text{ ms}$.

METHODS. *In vitro* motility assay. Myosin and actin were obtained from rabbit skeletal muscle and purified as described previously⁵. The myosin-coated surface was made by binding myosin monomers to a silicone-treated coverslip^{5,10}. Actin filaments were labelled with phalloidin-tetramethylrhodamine as previously described^{4,5}. The assay was done in a medium containing 25 mM KCl, 20 mM HEPES, pH 7.8, 5 mM MgCl₂, 2 mM ATP at 26–27 °C. To reduce photobleaching, 50 $\mu\text{g ml}^{-1}$ glucose oxidase, 9 $\mu\text{g ml}^{-1}$ catalase, 1.15 mg ml⁻¹ glucose and 0.5% 2-mercaptoethanol were added¹⁰. The ATPase activity of myosin heads interacting with sliding actin filaments was determined as described previously¹⁰. The ATPase activity per interacting head was obtained by dividing the measured inorganic phosphate (P_i) liberation rate per unit length of sliding actin filament by the number of myosin heads that can interact per unit length of actin filament. The number of interacting heads per μm length of actin filament was previously estimated from the density of myosin heads on the surface to be about 150 heads¹⁰. During sliding at zero load, the ATPase activity was $30 \pm 3 \text{ s}^{-1}$ per interacting head (s.e.m., $n = 6$). To measure the ATPase activity at zero velocity, surfaces



were prepared from mixtures of native myosin and NEM-treated myosin at a molar ratio of 4:1. The ATPase activity in this case was $6 \pm 0.5 \text{ s}^{-1}$ per interacting head (s.e.m., $n = 5$). The ATPase activities at intermediate velocities ($2\text{--}6 \mu\text{m s}^{-1}$), measured at low concentrations of ATP¹⁰, were almost the same as that at zero load.

TABLE 1 Parameters estimated on the basis of a simplified two-state on-off model

$f_c(\text{Hz})^*$	$V_{\text{ATP}}(\text{s}^{-1} \text{ per head})^\dagger$		$k_+(\text{s}^{-1})^\ddagger$	$k_-(\text{s}^{-1})^\ddagger$	$p_0^\S$	$F_0(\text{pN})^\parallel$	$\langle F \rangle(\text{pN})^\P$
5.1 ± 0.4	6.0 ± 0.5	Case 1	8.4 ± 0.4	23 ± 3	0.28 ± 0.03	1.5 ± 0.3	0.42 ± 0.1
		Case 2	23 ± 3	8.4 ± 0.4	0.72 ± 0.03	3.6 ± 0.4	2.6 ± 0.4

* Corner frequency of the power density spectra of the force fluctuation (s.e.m., $n=8$).

† ATPase activity at zero velocity (s.e.m., $n=5$) (see legend to Fig. 1.).

‡ Rate constants governing the transitions into and out of the on state, respectively.

§ Proportion of the time heads in the on state.

|| Force of a head in the on state.

¶ Mean force of a head.

total sliding was $>100 \text{ nm}$ and velocity was $>1 \mu\text{m s}^{-1}$. We compared the fluctuations of force during sliding with those obtained when the filament was nearly isometric.

The bending of the needle was measured by the system shown in Fig. 1*b*. To increase contrast, a nickel particle about $2 \mu\text{m}$ in diameter was attached to the tip of the needle. An image of the particle was projected on to a pair of photodiodes and the displacement of the needle was obtained from the differential output of the photodetector. The differential output was very sensitive to movement of the particle as already shown^{14,15,18} and was linear for motions from 0.1 nm to 100 nm (Fig. 1*c*). The lower limit of movement detection was $<0.1 \text{ nm}$.

To minimize deterioration of the time resolution due to the needle's mass and viscous drag in the solution, we used very fine microneedles $50\text{--}100 \mu\text{m}$ long and about $0.3 \mu\text{m}$ in diameter, considerably shorter and stiffer than in our previous experiments¹³. Needle stiffness (K_n) ranged from 0.09 to 0.5 pN nm^{-1} . Figure 1*d* shows the recorded motion of the needle with the nickel particle in a solution when sudden displacements were applied at the base of the needle. The rise time, $\tau(0\text{--}63\%)$, was 0.4 ms for the most flexible needle ($K_n = 0.09 \text{ pN nm}^{-1}$) used. Using this rise time the response time of the needle's motion driven by actomyosin interactions is estimated to be less than 1 ms (see Fig. 1*d* legend for details). The force produced by each myosin head is about 1 pN , corresponding to displacement of the needles by $2\text{--}11 \text{ nm}$, and the ATPase cycle occurring at $<50 \text{ s}^{-1}$ (ref. 19). The resolution of the force measurement system is high enough to detect the mechanical events coupled to the ATPase cycle of individual myosin heads.

Force fluctuations near isometric conditions

Minimal sliding was obtained by measuring the displacements of needles caused by a small number of myosin heads interacting with an actin filament. In this situation, large force fluctuations were observed immediately when the actin filament began to interact with the myosin-coated surface. Figure 2*a, b* shows the force fluctuations measured using needles with 0.15 and 0.5 pN nm^{-1} stiffness, respectively.

Figure 2*c* shows the power density spectrum of the data from Fig. 2*b*. The power density spectrum ($\Phi(f)$) nearly fits a lorentzian curve (solid line, $\Phi(f) \propto 1/[1+(f/f_c)^2]$), in which the power density decreases with the frequency squared (f^2) at high frequencies. The corner frequency (f_c), where $\Phi(f)$ is half the low-frequency asymptote, was $5.1 \pm 0.4 \text{ Hz}$ (s.e.m., $n=8$) when the pH of the medium was 7.8 . The f_c decreased to $<2 \text{ Hz}$ at pH 6.5 . Because the actomyosin ATPase activity decreases several-fold when pH is reduced from 7.8 to 6.5 ²⁰, the force fluctuations seem to be closely coupled to the ATPase cycle.

A lorentzian power spectrum would be expected if the actomyosin interactions occur randomly and independently of each other^{21,22}. In that case, the root-mean-square (r.m.s.) deviation from the mean force should be proportional to the square root of the mean force^{21,22}. Therefore we plotted the r.m.s. deviation ($\sqrt{\langle \delta F_N^2 \rangle}$) against the square root of the mean force ($\sqrt{\langle F_N \rangle}$) for experiments with varying numbers of interacting

heads (Fig. 2*d*). The linear relationship indicates that the force fluctuations are derived from independent, asynchronous force generators.

When we obtained the power density spectra ($\Phi(f)$) and the r.m.s. force fluctuations ($\sqrt{\langle \delta F_N^2 \rangle}$), the amplitudes were corrected for the intrinsic thermal motions of the needle and for attenuation of the motions due to the stiffness of myosin heads attached to actin. The stiffness of the attached myosin heads was measured by applying 50 or 100 Hz sinusoidal displacements at the base of the needle and measuring the displacement at the tip. This procedure was done for a series of experiments with different numbers of interacting heads (Fig. 3). Stiffness was proportional to force and under nearly isometric conditions was 0.012 pN nm^{-1} per pN of mean force.

Using the equipartition principle²³, the intrinsic thermal vibrations of the needle can be calculated from $\frac{1}{2}K_n\langle \delta X_n^2 \rangle = \frac{1}{2}(K_n + K_{mN})\langle \delta X_n'^2 \rangle = \frac{1}{2}k_B T$, where $\langle \delta X_n^2 \rangle$ and $\langle \delta X_n'^2 \rangle$ are the mean squared thermal deflections of the needle in the absence and presence of actomyosin interactions, respectively, K_n is the stiffness of the needle, K_{mN} is the total stiffness of N actomyosin attachments and k_B is Boltzmann's constant. Let the attenuation factor $\alpha = K_n/(K_n + K_{mN})$, then $\langle \delta X_n'^2 \rangle = \alpha \langle \delta X_n^2 \rangle$. If the observed deflections of the needle during force production are $\delta X(t)$, then the r.m.s. deviation corrected for the thermal motion is given by $D = \sqrt{\langle (\delta X^2(t) - \alpha \langle \delta X_n^2 \rangle) \rangle}$. This r.m.s. deflection is proportional to the fluctuating force $\sqrt{\langle \delta F_N^2 \rangle}$ caused by the acto-myosin interactions by $D = \sqrt{\langle \delta F_N^2 \rangle} / (K_n + K_{mN}) = (\alpha / K_n) \sqrt{\langle \delta F_N^2 \rangle}$. Thus the true r.m.s. force fluctuation corrected for thermal vibrations and attenuation by attachments, was obtained from $\sqrt{\langle \delta F_N^2 \rangle} = K_n D / \alpha = (K_n / \alpha) \sqrt{\langle (\delta X^2(t) - \alpha \langle \delta X_n^2 \rangle) \rangle}$. Similarly, the power density spectrum of force fluctuations was obtained as $\Phi(f) = (K_n^2 / \alpha^2) (\phi_x(f) - \alpha \phi_{x_n}(f))$, where $\phi_x(f)$ and $\phi_{x_n}(f)$ were the power density spectra of the needle fluctuations during force production and in the absence of actomyosin interactions, respectively. For a typical experiment with 5 pN of mean force, $K_{mN} = 0.06 \text{ pN nm}^{-1}$. If the needle stiffness is 0.15 pN nm^{-1} , then $\alpha = 0.71$. The correction of $\sqrt{\langle \delta F_N^2 \rangle}$ for thermal motions is about 3% .

The force fluctuations are analysed in terms of two models. First we analyse the data using a simplified model, in which the myosin heads randomly take on and off states. The force is constant in the on state and zero in the off state. This simple analysis provides an outline of the relationship between the mechanical and ATPase cycles. Second, we present the data as a computer simulation using the model of A. F. Huxley¹⁷.

In the simple on-off model, the power density spectrum is lorentzian and the r.m.s. force fluctuation is expressed by

$$\sqrt{\langle \delta F_N^2 \rangle} = \sqrt{(1-p_0)F_0} \sqrt{\langle F_N \rangle} \quad (1)$$

where p_0 is the proportion of the time heads are in the on state and F_0 is the force of a head in the on state^{21,22}. The r.m.s. force fluctuation ($\sqrt{\langle \delta F_N^2 \rangle}$) is proportional to the square root of the mean force ($\sqrt{\langle F_N \rangle}$) (Fig. 2*d*) as expected from this equation. Therefore, the slope of the line in Fig. 2*d* gives the value of

$\sqrt{(1-p_0)F_0}$. The value of p_0 can be obtained from the corner frequency f_c of the power spectrum and the ATPase rate (V_{ATP}) as follows. The rate constants governing the transitions into (k_+) and out of (k_-) the force-generating state are related to f_c and V_{ATP} by the relations $k_+ + k_- = 2\pi f_c$ and $V_{ATP} = (1/k_+ + 1/k_-)^{-1}$. These two equations can be combined yielding a quadratic equation for k_+ and k_- involving V_{ATP} and f_c . Two solutions of the quadratic equation are possible depending on whether k_+ is the higher or lower of the two rate constants; p_0 is given by $k_+/(k_+ + k_-)$. F_0 can be determined from $\sqrt{(1-p_0)F_0}$ using p_0 . The mean force produced by one myosin head (F) is given by $p_0 F_0$. The number of heads (N) involved in the force generation can be also determined as $N = \langle F_N \rangle / \langle F \rangle$. The value of f_c averaged 5.1 Hz, indicating that $k_+ + k_- = 32 \text{ s}^{-1}$ and V_{ATP} was 6 s^{-1} (Table 1). The two solutions for k_+ and k_- are given in Table 1 along with the corresponding values of p_0 , F_0 and $\langle F \rangle$.

In several experiments, the mean force ($\langle F_N \rangle$) was less than 2.6 pN (Fig. 2d). If k_+ is the smaller rate constant, $\langle F \rangle = 2.6 \text{ pN}$ (Table 1, case 2), so N would be < 1 . As it is unlikely that $N < 1$, case 1 is probably the correct solution and thus the mean force per myosin head (F) is 0.4 pN (Table 1), which is several times smaller than the value expected for a muscle fibre, 1–2 pN per head¹⁷. It is likely that the force vectors of myosin heads randomly oriented on the surface are not all parallel to the axis

of actin filament, decreasing the axial force exerted on the filament. If this is correct, the true force would be larger than 0.4 pN, probably about 1 pN.

As the on-off model is too simple to explain the actual events in force generation, we also analyse the data by a computer simulation based on A. F. Huxley's model of the cross-bridge cycle¹⁷. That model is also a two-state, on-off scheme but the rate constants for transitions between the states varied with the mechanical strain (x) of the attachment. The strain dependence of the rate constants was designed to explain many facets of muscle mechanics and energetics. The force fluctuations and the power density spectrum under isometric conditions could be simulated well by this model (Fig. 2c). To fit the results, the parameters of the model were set to the following values: f_1 (on rate at $0 < x < 10 \text{ nm}$) = 1 s^{-1} , g_1 (off rate at $0 < x < 10 \text{ nm}$) = $2x \text{ s}^{-1}$; g_2 (off rate at $x < 0$) = 390 s^{-1} .

With both models adjusted to fit the experimental data, each on-off cycle could be related to the splitting of one ATP molecule. This result suggests that during isometric force generation, chemo-mechanical coupling is one-to-one.

Force fluctuation during sliding

When the length of actin filaments in contact with the myosin-coated surface was relatively long (0.5–3 μm) and the generated

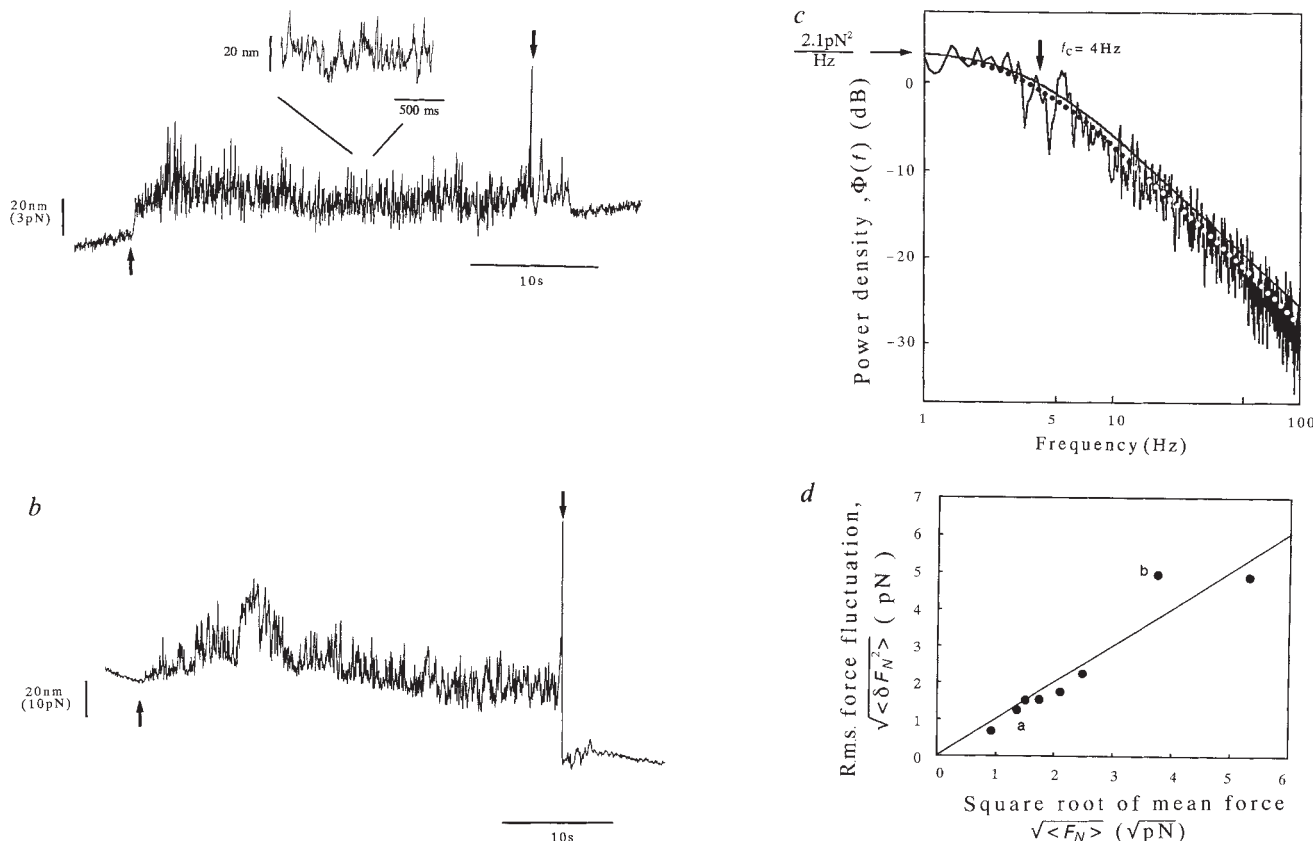


FIG. 2 *a, b*, Force fluctuation near isometric conditions. The actin filaments were caught by needles with stiffness values of 0.15 pN nm^{-1} in *a* and 0.5 pN nm^{-1} in *b* and then brought into contact with the myosin-coated surface in the mobility assay medium containing ATP at 26–27 °C. The actin filaments moved on the surface and started to pull the needle at the time indicated by '↑' and were detached from the surface at '↓'. Noise seen at the beginning and end of each recording is thermal vibration of the needle alone. Changes of the base lines (zero-force level) are due to mechanical and thermal drifts. Insertion is part of the trace expanded by 15 times on the timescale. When the actin filaments were brought into contact with the surface and stretched in the absence of ATP (rigour), the vibration of the needle almost completely disappeared due to stiff rigor actomyosin attachments (data not shown). *c*, The power density spectrum ($\Phi(f)$) of the force

fluctuations shown in *b*. The data were corrected as described in the text for thermal vibrations of the needle and attenuation due to actomyosin attachments before plotting. The solid line is a theoretical Lorentzian curve based on the on-off model ($\Phi(f) = \Phi(0)[1/(1 + (f/f_c)^2)]$). The curve was fitted to the data (before logarithmic transformation by a least-squares method). The values of f_c and $\Phi(0)$ used were 4 Hz and $2.4 \text{ pN}^2 \text{ Hz}^{-1}$, respectively. The dotted line is the power density spectrum of the force fluctuations obtained from a computer simulation based on the model of A. F. Huxley¹⁷. Details of the simulation will be published elsewhere. The parameters used are given in the text. *d*, The relationship between the r.m.s. amplitude of the force fluctuations ($\sqrt{\langle \delta F_N^2 \rangle}$) and the square root of the mean force ($\sqrt{\langle F_N \rangle}$). Points labelled *a* and *b* correspond to data shown in *a* and *b*, respectively.

forces were high (20–100 pN), the actin filaments moved 150–600 nm at average velocities of $0.15\text{--}4\ \mu\text{m s}^{-1}$. These velocities correspond to 1.6–45% of the velocity at zero load which is about $9\ \mu\text{m s}^{-1}$ (27°C). When the generated force was balanced by the elastic restoring force of the needle and the velocity decreased to be zero, the force quickly dropped to a very low or sometimes zero value. This decreased stability when the ratio of interacting filament length to the needle stiffness was large was probably caused by the larger displacements resulting from force fluctuations compared with the relatively small displacements in the nearly isometric condition.

At the average velocities less than $0.2\ \mu\text{m s}^{-1}$ the force fluctu-

ated as much as under isometric conditions (Fig. 4a). But the force developed quite smoothly at velocities $>1\ \mu\text{m s}^{-1}$ (Fig. 4b, c). The data during sliding were corrected for intrinsic thermal fluctuations of the needle and attenuation by the actomyosin attachments as described earlier. During sliding, the stiffness of the actomyosin attachments increased as force developed; $K_{mN}(t) = 0.006\ \text{pN nm}^{-1}\ \text{pN}^{-1}\langle F_N(t)\rangle$ (Fig. 3). The force attenuation was calculated at each time point as $\alpha(t) = K_n/(K_n + K_{mN}(t))$ (Fig. 4, bottom row). The average r.m.s. force fluctuations were then calculated as $\sqrt{\langle \delta F_N^2 \rangle} = (K_n)\sqrt{1/J \sum_{i=1}^J (\delta X(t_i)^2 - \alpha(t_i)\langle \delta X_N^2 \rangle)/\alpha(t_i)^2}$, where J was the sampling number and set to be 1,000. Using this procedure, the

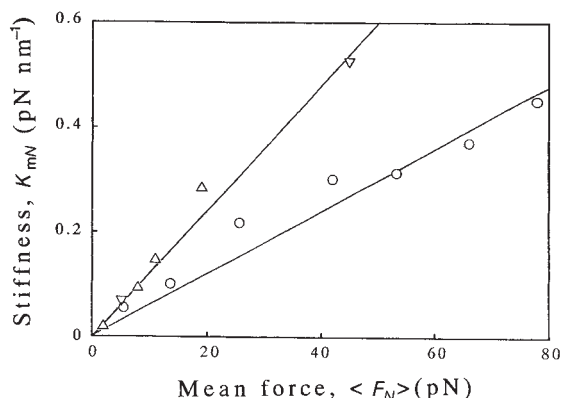


FIG. 3 The stiffness of myosin heads attached to actin near isometric conditions (Δ , ∇) and during sliding ($\circ$). For nearly isometric conditions, 10 nm sinusoidal displacements were applied to the base of the needle at frequencies of 50 (Δ) or 100 Hz (∇). The stiffness, obtained by measuring the amplitude of the sinusoidal displacement at the tip of needle with the FFT analyser, was $0.012\ \text{pN nm}^{-1}$ per pN of mean force. If the mean force $\langle F \rangle$ and the maximum force F_0 of a head are 0.42 and 1.5 pN as shown in the text (also see Table 1), the mean and the maximum stiffness per head would be 0.005 and $0.018\ \text{pN nm}^{-1}$, respectively. These values are much smaller than the stiffness expected from the instantaneous force–extension curve of muscle fibres²⁴ probably because at 27°C , the recovery time for force after a quick perturbation is much shorter than the 10–20 ms period of the imposed sinusoidal oscillation. During filament sliding at velocities of $1\text{--}6\ \mu\text{m s}^{-1}$, the stiffness was obtained by directly measuring the amplitude of sinusoidal motion at the tip using 30 nm, 120 Hz sinusoidal displacements at the base of the needle. For example, the sinusoidal amplitude decreased from 30 to 6 nm as the force increased from 0 to 80 pN (the maximum displacement was 600 nm) when the needle stiffness was $0.15\ \text{pN nm}^{-1}$. Each plotted point ($\circ$) indicates a value averaged for about every 10 pN during force development. Stiffness during sliding was $0.006\ \text{pN nm}^{-1}$ per pN of mean force, about one-half that in nearly isometric conditions.

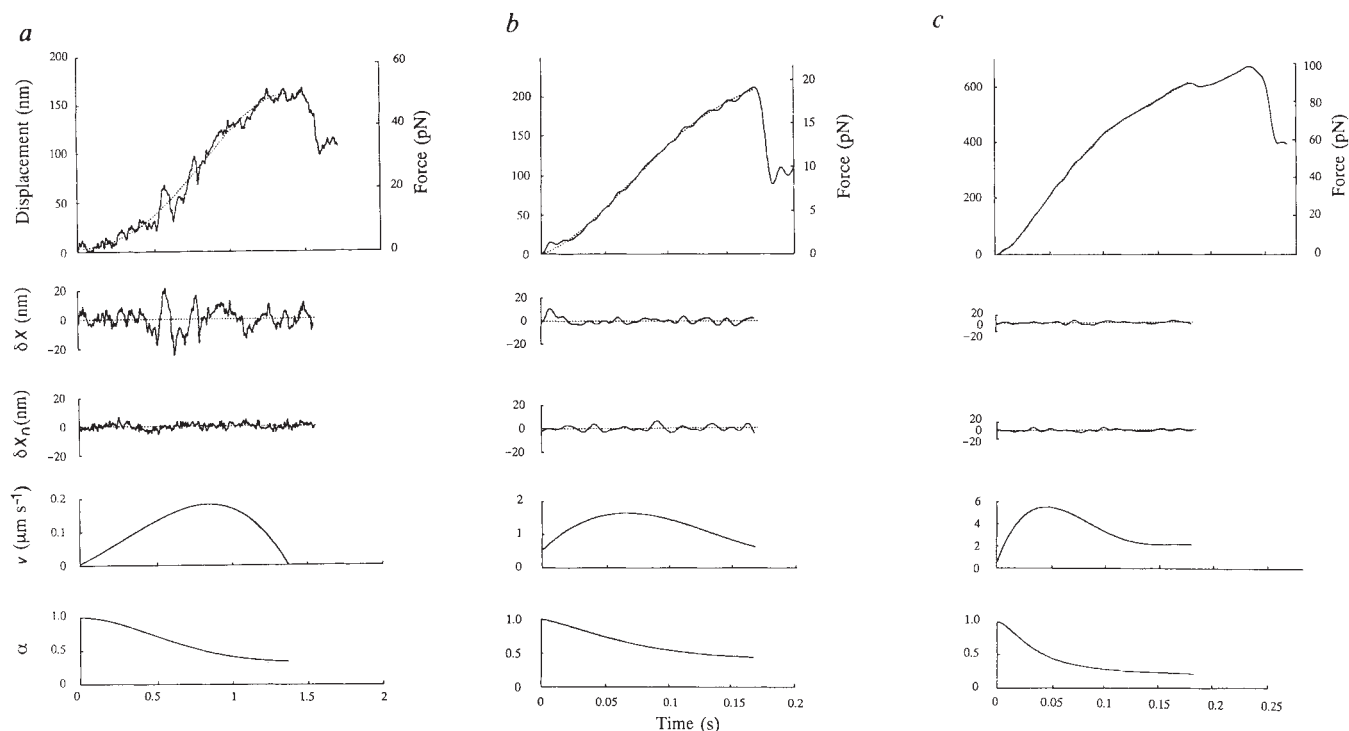


FIG. 4 Force fluctuations during the sliding at low (a), middle (b) and high velocities (c). Top row, the time course of force generation after an actin filament contacts the myosin-coated surface. Needle stiffness was $0.32(a)$, $0.09(b)$ and $0.15\ \text{pN nm}^{-1}(c)$. The broken lines are averaged curves ($\langle X \rangle$) obtained by fitting a fifth order polynomial to the displacement trace. Second row, the deviations ($\delta X = X - \langle X \rangle$) of the needle position from the averaged curves. Third row, thermal vibrations of the needle alone ($\delta X_n = X_n - \langle X_n \rangle$).

The ordinates in second and third rows are scaled up 1.5-fold compared with the top ones. Fourth row, the time courses of the sliding velocities. Bottom row, the values of the attenuation factor α at each time during sliding, calculated using the stiffness of actomyosin attachments ($K_{mN}(t)$) as described in the text. The sampling time was set to 2.5 ms for *a* and 0.25 ms for *b* and *c*.

ratios of r.m.s. force fluctuations to mean force averaged over the sliding period were $\sqrt{\langle \delta F_N^2 \rangle} / \langle F_N \rangle = 0.036$ for Fig. 4b and 0.023 for Fig. 4c. These values are 10- and 6-fold smaller than those obtained under isometric conditions at the same mean forces (0.35 at 10 pN and 0.14 at 50 pN; Fig. 2d). These results indicate that the myosin heads produce force much more smoothly during sliding than under isometric conditions. Therefore, the proportion (p_0) of the time the heads are in the force-generating state seems much greater than that under isometric conditions, 0.28 (Table 1).

In the on-off model, for example, rearrangement of equation (1), noting that $F_0 = \langle F \rangle / p_0$ gives $\sqrt{\langle \delta F_N^2 \rangle} / \langle F_N \rangle = \sqrt{(1-p_0)/p_0} / \sqrt{N}$. Taking $\langle F_N \rangle$ to be the overall average of force during the force development and N as (maximum mean force at the end of each trace)/0.4 pN, p_0 during the sliding was found to be 0.94 for Fig. 4b and 0.88 for Fig. 4c. In these estimations, N was assumed to be maximal during the sliding period. If N was smaller, p_0 would be greater.

Thus, the results imply that myosin heads produce an almost constant force for most (probably 90%) of the ATPase cycle time during moderate to high velocity sliding.

Discussion

Muscle contraction is generally thought to be caused by stochastic and independent attachment-detachment cycles between myosin heads and actin¹⁷. Most models assume that one active attachment-detachment cycle corresponds to hydrolysis of one molecule of ATP^{17,19,24-28}. We have observed large fluctuations during isometric force generation that agree well with the stochastic, independent and one-to-one coupling hypotheses. Our data thus provide direct experimental substantiation of these basic principles.

But this did not apply when the filaments were sliding. At velocities greater than about $1 \mu\text{m s}^{-1}$, the force fluctuations almost completely disappeared (Fig. 4b, c). This effect was not caused by limited time resolution of the apparatus because the response time of the needles was <1 ms, fast enough to detect the mechanical impulses as often as the actomyosin hydrolysed ATP (about 30 s^{-1}). Also, it is unlikely that smooth force development is due to a precisely regular sequence of mechanical impulses from the interacting molecules. The analysis implies that myosin produces an almost constant force for about 90% of the ATPase cycle time during sliding, in disagreement with the generally accepted hypotheses²⁹⁻³¹. In these hypotheses, it is assumed that one working stroke (about 10–15 nm) of a myosin head occurs during hydrolysis of one molecule of ATP, independent of the load^{17,24,32}. Therefore, the duration of the

working stroke, obtained as (length of the working stroke, 10–15 nm)/(sliding velocity, v), decreases as the velocity increases. The ratio of the working stroke time to ATPase cycle time, that is, p_0 , also decreases. For example, at $v = 4 \mu\text{m s}^{-1}$ the duration of the working stroke would be 2.5–4 ms and $p_0 = 0.08$ –0.13. These values are about 10-fold smaller than the values obtained here. If the working stroke is >10 –15 nm, then the proportion of cycle time spent in the force-generating state would be increased. But it is unlikely that the working stroke produced by a single attachment-detachment cycle of actomyosin is much greater than the size of a myosin head (~ 20 nm)³². Therefore, the large value of p_0 suggests that multiple working interactions between actin and myosin correspond to each ATPase cycle during sliding at velocities of $>1 \mu\text{m s}^{-1}$ or as the time while a myosin head spends close to an interacting site of actin is short (<5 ms within 5 nm).

The problem of whether coupling between the biochemical and mechanical reactions is rigidly determined has been controversial, as we recently reported that the sliding distance of an actin filament per ATP molecule hydrolysed in single sarcomeres without Z-lines³³ and in the myosin-surface motility assay¹⁰, is at least 60–200 nm. This sliding distance is several times larger than expected on the basis of rigid chemo-mechanical coupling, in which one working stroke corresponds to each ATPase cycle independent of the load and velocity^{17,24-32}. As this is an essential point for the mechanism of chemo-mechanical energy transduction, it has attracted a good deal of attention not only regarding muscle^{11,12,20,30,34-41} but also in other forms of motility such as microtubule-based motor proteins^{26,41-45} and the rotary motor of bacterial flagella^{46,47}. In most cases, however, the movements have been analysed essentially at zero load. A serious problem with these previous studies is that at zero load, the energy required to induce sliding is negligible^{33,37} so every interaction between the motor protein and its substrate filament need not be truly active. This ambiguity is avoided in these experiments, as chemo-mechanical coupling was examined at various loads and velocities.

In conclusion, the results imply that the mechanical to chemical coupling ratio is one-to-one in isometric conditions but many-to-one during filament sliding. We propose that when work is done at higher velocity, the myosin head directly uses the free energy liberated by the hydrolysis of a single ATP molecule for many working strokes. Alternatively, the energy could be efficiently transferred through actin to multiple heads, to generate multiple working strokes. □

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Quantized velocities at low myosin densities in an *in vitro* motility assay

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An *in vitro* motility assay has been developed in which single actin filaments move on one or a few heavy meromyosin (HMM) molecules. This movement is slower than when many HMM molecules are involved, in contrast to analogous experiments with microtubules and kinesin. Frequency analysis shows that sliding speeds distribute around integral multiples of a unitary velocity. This discreteness may be due to differences in the numbers of HMM molecules interacting with each actin filament, where the unitary velocity reflects the activity of one HMM molecule. The value of the unitary velocity predicts a step size of 5–20 nm per ATP, which is consistent with the conventional swinging crossbridge model for myosin function.

THE mechanism by which the chemical energy released by ATP hydrolysis is converted to relative sliding between actin filaments and myosin filaments in contracting muscle has remained a matter of long-standing debate. The conventional swinging crossbridge theory¹ proposes that such a sliding is driven by cycles of sequential attachment of a myosin head to an actin filament, a change in its attachment angle (giving rise to the power stroke), followed by its release. Each step of the mechanical cycle is tightly coupled to a step in the ATP hydrolysis cycle. According to this model the translocation distance of an actin filament per ATP hydrolysis cycle (step size) must be less than 40 nm, the maximum possible stroke size of a myosin head (20 nm long) swinging through an arc of 180°. Recently, the swinging crossbridge theory has been seriously challenged by Yanagida *et al.* who estimate a step size much larger than 40 nm per ATP^{2,3}. These estimates prompted new proposals⁴ and revivals of other mechanisms^{5,6} for the chemomechanical energy transformation in which the step size is not limited by the physical size of myosin heads. Meanwhile, contradictory reports of step sizes smaller than 40 nm per ATP have accumulated^{7–10}.

All of these measurements of large and small step sizes can be criticized for their dependence on a number of uncertain assumptions. Such uncertainties are inherent in experiments which involve large numbers of actin and myosin molecules. Just as study of the conductance properties of single membrane channels has advanced understanding of the molecular mechanism of regulated membrane permeability, observation of movements produced by individual myosin molecules in an *in vitro* motility assay might help clarify the mechanism of actomyosin-dependent motility.

It has been estimated that the proportion of the duration of the force-generating state or the strongly bound state to the total ATPase cycle time (duty ratio) of skeletal muscle myosin is small^{9,10}. Thus, when an actin filament is interacting with a small number of myosin heads fixed to a glass surface in an *in vitro* motility assay, in the presence of ATP the filament would be mostly only held to the surface by the heads in the weakly bound states, undergoing brownian-like motion. Such a filament would

rapidly dissociate from the surface without making continuous movement, if not retained by some other force. In a previous study¹¹ a threshold density of HMM on the surface was found, below which actin filaments rapidly diffused away from the surface. But inclusion of the inert polymer, methylcellulose, in the motility buffer restricts lateral diffusion of actin filaments without significantly affecting their forward motion driven by myosin heads^{9,12}. This effect of methylcellulose enabled us to observe continuous movement of actin filaments over HMM surfaces below the previously measured threshold density in the absence of methylcellulose⁸. We have now extended these observations by analysing the properties of actin sliding at such low densities of HMM that many actin filaments, though held to the surface by methylcellulose, fail to slide owing to the lack of HMM molecules available to interact with.

Quantized velocities

If the HMM density is decreased such that many filaments are unable to interact with any HMM molecules, other filaments should have only one or a very small number of HMM molecules to interact with. Under such conditions, many filaments showed axial brownian movement, whereas others moved slowly and unidirectionally in an ATP-dependent manner, probably through interaction with a small number of HMM molecules. To characterize these movements, the centroid positions of fluorescence images of the actin filaments were determined for every video frame.

In this way, displacement-time curves were generated, which consist of intervals of axial brownian movement and intervals of up to several seconds of unidirectional sliding movement (Fig. 1). The intervals of unidirectional movement appeared to be further divided into short segments of a second or less during which the velocity remains relatively constant. We interpret each short segment of unidirectional movement as movement driven by one set of HMM molecules interacting with the filament, where different velocities of different segments reflect changes in the number of HMM molecules interacting with the filament during that period. To assign a velocity to the segments of brownian movements and active movements, we fitted each segment with a line. The slopes (velocities) of the fitted lines were used to obtain a distribution of velocities (Fig. 2b–d). The polarity of the velocities was determined such that the direction of the active movement is positive. As expected, the distribution has a large peak around zero decreasing towards larger positive and negative velocities, representing the brownian movement. Strikingly, in the presence of HMM the positive half of the brownian distribution is superimposed with progressively smaller peaks separated by a regular interval. Fast Fourier transform analysis demonstrated that the positive velocities have a periodicity of about $0.31 \mu\text{m s}^{-1}$ (Fig. 2e). These regularly spaced peaks are observed only for the positive velocities.

The same analysis was done on five independent sets of data (Table 1), one of which is a control obtained using surfaces not coated with HMM (Fig. 2a). The regularly spaced peaks of positive velocities were observed in all four data sets of movements over low density HMM surfaces. The periodicity was $0.31\text{--}0.35 \mu\text{m s}^{-1}$ in three data sets (average $0.33 \mu\text{m s}^{-1}$) and $0.50 \mu\text{m s}^{-1}$ in one. Why one preparation of HMM gave a larger periodicity is unknown. It may not be surprising, however, as a comparable difference in the maximum sliding velocity over

high-density HMM surfaces has been observed among different proteolytic preparations of HMM (Y. Toyoshima, manuscript in preparation).

It was important to eliminate the possibility that the observed quantization of velocity was due to artefacts generated by the data processing procedure. The centroid position of the image of actin filaments in each video frame was calculated from digitized intensity data of video pixels. If, for example, the centroid was fixed to a single position in each video pixel, only discrete displacements would be allowed, and such artefactually discrete displacements might result in artefactual creation of quantized velocities. But this problem was avoided by using a computer program that determines the centroid position to a subpixel level (except for data set 1). In addition, to eliminate the possibility that any part of the optical, video or image analysis system might give rise to such an artefact, the same analysis was done on data recorded using $\times 63$ and $\times 100$ objectives and the same result was obtained for these two sets of data. Finally, the regularly spaced peaks were not observed for the brownian movements (velocities in Fig. 2a and the negative velocities in Fig. 2b). This in particular demonstrates that the quantized velocities reflect properties of the active movement, rather than that of the data acquisition or processing procedures. Sometimes when the data set size was small, minor peaks were found at apparently random positions in the negative velocities (for example Fig. 2c, d), probably arising from statistical variation in sampling.

What is the origin of the quantized velocities? The Reynold's number of actin filaments in a typical *in vitro* movement assay is very small, reflecting the infinitesimal contribution of inertia to their motion relative to the effect of viscosity. Yet the viscous drag on actin filaments is negligible compared with the force generated by a single myosin head^{2,13}. This means that actin filaments move at a velocity limited by kinetic properties of the force-generating process, only when under the influence of at least one strongly bound head^{2,13}. Second, if the duty ratio for

TABLE 1 Fast Fourier transform analysis of sliding velocities from different myosin preparations

Data set	Myosin preparation	Unitary velocity* ($\mu\text{m s}^{-1}$)	Number of filaments†	Frames fitted with lines/total frames
1‡§	A	0.35	26	2,326/7,712
2 ¶	B	0.50#	54	6,663/12,777
3§¶	C	0.31	32	6,553/12,534
4§¶	C**	0.34	48	11,698/19,458
5§¶	none††	none	25	4,598/10,811

* Unitary velocity was determined by Fast Fourier transform analysis.

† Lengths of filaments were between 1.2 and 2.4 μm .

‡ Centroid positions of filament images were determined using JAVA (Jandel Scientific, Corte Madera, California).

§ Observation was made using a Zeiss Planapo $\times 63$ objective.

|| Observation was made using either Zeiss Planapo $\times 63$ or $\times 100$ objective.

¶ Centroid positions were determined using Optimas (BioScan, Edmonds, Washington). Unlike JAVA, this program does not round the centroid position to a single value in each pixel.

Fast Fourier transform analysis of data from this HMM preparation suggested that it was a mixture producing a major unitary velocity of $0.50 \mu\text{m s}^{-1}$ and a minor one at $0.36 \mu\text{m s}^{-1}$.

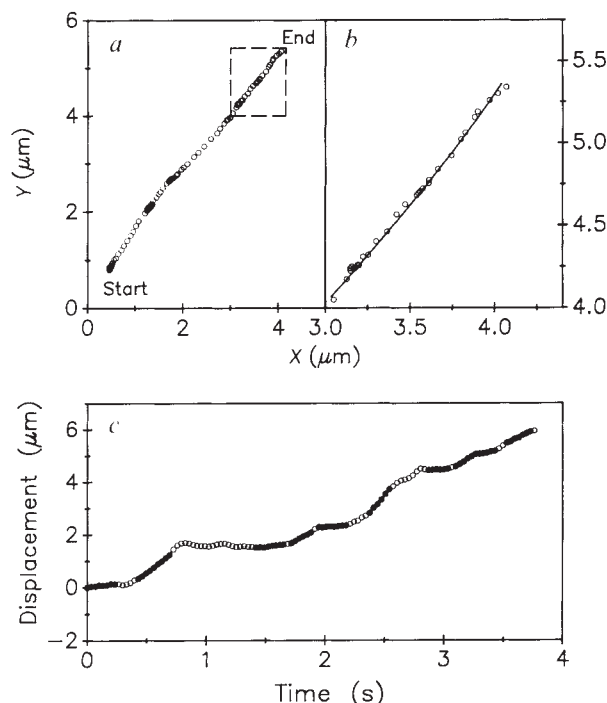
** HMM was prepared from the same batch of myosin as data set 3, except that myosin was used fresh without storage at -20°C in 50% glycerol.

†† Surfaces were not coated with HMM.

myosin heads is small^{9,10} and if the total number of heads interacting with an actin filament is small, the probability of more than one head being in a force-generating state is very low. These two considerations allow the deduction that the displacement of an actin filament generated by each myosin head will be essentially additive when the total number of heads interacting with the filament is significantly smaller than the

FIG. 1 Movement of an actin filament over a sparse surface of HMM. This filament (1.7- μm long) belongs to data set 2 (Table 1). a, Centroid trajectory of the actin filament shown in the x-y plane. The circle represents the centroid position in each video frame. b, Higher magnification of the boxed area in a. To eliminate small lateral components of the displacements, the centroids were projected onto the polynomial fitted curve shown by the line. Displacement of the filament (c) was calculated from the projected centroids. Each series of closed circles indicates a segment fitted with a straight line (see below). Slopes of these lines were used for the construction of velocity distribution. Other segments (open circles) were not well fitted with lines (standard deviation after linear regression exceeded a conservatively set limit) and discarded.

METHODS. Actin and HMM were prepared from rabbit back muscle and the actin-activated Mg-ATPase activity was measured in motility buffer¹¹. *In vitro* movement of rhodamine-phalloidin labelled actin filaments over surfaces sparsely coated with HMM was done at 30°C as described⁸ with the following modifications. The surfaces were prepared by incubation with $1.5\text{--}2.0 \mu\text{g ml}^{-1}$ HMM solution for 2 min. Methylcellulose was used at a concentration of 1.8%. The viscosity of this solution was dependent on the shear rate (see ref. 9), and was 480 centipoise (cP) at 11.25 s^{-1} and 405 cP at 45 s^{-1} . Fluorescence images of actin filaments were recorded on optical discs, and each video frame was digitized. Using video processing software, each frame was treated with a low pass filter (7×7 convolution) and the perimeter of the filament image was determined with reference to a set intensity threshold. The centroid position was then calculated from the perimeter. With this method, the position of actin filaments fixed to polylysine-coated surfaces could be determined to $\pm 14 \text{ nm}$ (s.d.) in each frame. The centroid trajectory of each actin filament was fitted with a fourth order polynomial curve and each centroid was projected perpendicularly onto the curve, a minor adjustment to reduce the lateral displacements. Each longitudinal displacement between successive video frames, calculated from the difference of the projected centroids, was scored for direction (with or against the net displacement), summed and plotted against time. This displacement-time curve was fitted with a series of straight lines. Each line was determined as follows. First, a seven-frame (200 ms) segment was identified whose standard deviation after linear regression was less than



a set limit ($0.0175 \mu\text{m}$). After the window was further shifted in the vicinity to minimize the standard deviation, the segment was extended at both ends until the standard deviation exceeded the limit. Then a terminal frame was deleted from both ends and the linear regression line of the resultant segment was used as the fitted line for that segment. The same procedure was repeated until the end of the record.

inverse of the duty ratio. Therefore, the average sliding velocity of actin filaments interacting with two HMM molecules (four heads) will be almost twice as fast as those interacting with one HMM (two heads) (Fig. 3). We suggest that this is why the observed movements distribute around integral multiples of a unitary velocity where the velocity of the slowest peak or the unitary velocity reflects the activity of one HMM molecule interacting with one actin filament.

As the number of heads interacting with a filament increases, the probability of overlaps between force-generating states of the heads should become significant. In a simplified model, myosin heads act independently and stroke at a constant speed

of d/t_s during the strongly bound state, where d is the step size and t_s is the duration of the strongly bound state. Then the average sliding velocity (v_{av}) of a filament interacting with N heads is proportional to the probability of being strongly bound to at least one force-generating head, and is given by $v_{av} = (d/t_s) \times (1 - (1 - t_s/t_c)^N)$, where t_c is the cycle time of the ATPase per head. According to this model, if the duty ratio (t_s/t_c) is 0.05 (ref. 9), v_{av} of a filament interacting with two, three, four and five HMM molecules (two motor units per molecule) will be 1.90, 2.72, 3.45 and 4.11 times as fast as that of a filament interacting with one HMM, respectively. When $N = \infty$, v_{av} reaches d/t_s , which is $t_c/(2 \times t_s) = 10$ times faster than that of a filament interacting with one HMM.

It should be noted that quantized velocities are not necessarily inconsistent with the view of Yanagida *et al.*³. According to their model, a strongly bound state makes up most if not all of the duty cycle, where myosin heads remain associated with actin filaments during displacements up to 100 nm. Thus, multiple heads interacting with a single filament implies the simultaneous active interaction of nearly all of those heads. Therefore, if filament velocity is determined by the balance of the total active force and the external resistive load, summation of active forces generated by each myosin head would result in discrete velocities of actin filaments.

This hypothesis can be tested as the external resistive load on an actin filament should be proportional to the filament length. If the quantization of the velocity reflects the additive contributions of force from small integral numbers of heads, then the magnitude of the unitary velocity should decrease with increasing filament length. But the periodicity of the velocity distributions of shorter, medium and longer filaments were the same (Fig. 2b–d), which argues against external resistive load limiting the sliding velocity. Furthermore, we have consistently observed that increasing the concentration of methylcellulose does not slow down the active movement even when the density of heads on the surface is very low^{9,12}, suggesting that the increased viscosity in this experiment is imposing negligible levels of load on the movement. Another interesting point with the comparison of velocity distributions of filaments of different lengths (Fig. 2b–d) is that the height of the slowest peak was relatively larger in the velocity distribution of shorter filaments. This result is consistent with the idea that the quantized velocities reflect the difference in the numbers of HMM molecules interacting with the filament, because longer filaments should have a smaller probability of interacting with a smaller number of HMM molecules.

The most plausible explanation, therefore, for the quantized velocities is that the displacements, not forces, generated by each HMM interacting with one actin filament are additive and each quantized velocity reflects motile activity of integral numbers of unloaded HMM molecules. Such additive nature of displacements demands the duty ratio for HMM be small. Our recent estimate of this ratio, 0.05 (ref. 9), is consistent with the unitary velocity of $0.33 \mu\text{m s}^{-1}$ measured here, within a factor of 2. The maximum sliding velocity on a dense surface of HMM molecules under the identical conditions was found to be $7 \mu\text{m s}^{-1}$ (ref. 9), which when multiplied by the duty ratio of 0.05×2 (because each HMM has two heads) yields $0.7 \mu\text{m s}^{-1}$.

It should be noted that the peaks of positive velocities seem tighter than the distribution of velocities around zero (Fig. 2b). The latter probably represents brownian movements of free actin filaments. The average travelling distance of actin filaments in brownian motion should be proportional to the square root of travel time. Therefore, if the duty ratio is small, restriction of brownian movement of actin filaments by heads in the strongly bound state should not significantly restrain the distribution of velocities due to brownian movements. That is, the time spent in the strongly bound state is too short to explain the tightness of the peaks of positive velocities. Presumably, heads in the weakly bound states also contribute to the suppression of

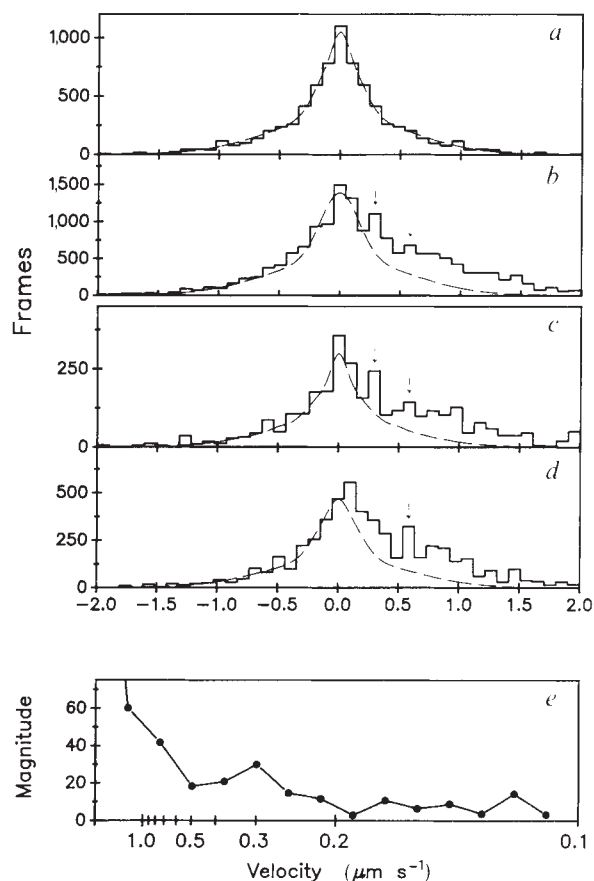


FIG. 2 Velocity distribution of movement of actin filaments over sparse HMM surfaces. *a*, No HMM control. The direction of the brownian movements could not be determined with respect to the structural polarity of the actin filament. Therefore, all brownian velocities were accumulated on one side and are shown as a symmetric distribution about zero. *b*, The velocity distribution of movements of medium length filaments (between 1.2 and 2.4 μm , average 1.9 μm) over low density HMM surfaces. *c*, The velocity distribution of shorter filaments (between 1.0 and 1.7 μm , average 1.4 μm). *d*, The velocity distribution of longer filaments (between 2.2 and 2.6 μm , average 2.4 μm). The dashed lines are fits to the distribution of negative velocities in each data set, converted to a symmetric distribution about zero to demonstrate the approximate contributions of brownian movements and active movements to the velocities on the positive side of the distribution. Arrows indicate the position of unitary and $2 \times$ unitary peaks. *e*, Fast Fourier transform analysis of the velocities between 0 and $2.0 \mu\text{m s}^{-1}$ shown in *b*. METHODS. For the no HMM control, data set 5 (Table 1) is shown. For movements over low density HMM surfaces, data sets 3 and 4 were combined (these two data sets were derived from the same batch of myosin and have the same periodicity). Except for *a*, those filaments which did not make net displacements larger than several micrometers in 10 s were discarded because the polarity of such filaments could not be judged. The velocity distributions were constructed from slopes of lines (weighted with their lengths) which fitted segments of displacement curves of each filament. Changing the limit for the standard deviation for line fitting to either 0.0125 or 0.025 μm did not affect the position of the peaks.

brownian movements of actin filaments. In fact, relaxed muscle fibres at low ionic strength, conditions in which myosin heads are in a weakly bound state, have some stiffness¹⁴. Muscle fibres in the presence of ATP γ S, an ATP analogue which is supposed to trap the majority of myosin heads in a weakly bound state, also show significant stiffness¹⁵. Consistent with these earlier reports, the actin filaments on surfaces moderately coated with HMM (5 times denser than those used in the present study) showed no noticeable brownian nor active movements in the presence of 5 mM Mg-ATP γ S and 1.8% methylcellulose in the motility buffer (data not shown), indicating significant interaction between actin and myosin. In the absence of methylcellulose, actin filaments rapidly diffused away from the surface on the addition of Mg-ATP γ S, indicating that this interaction is relatively weak. Thus, this weakly bound state restricts brownian motion in our *in vitro* assay. The tightness of the peaks of positive velocities is most easily explained in this way and is consistent with a small duty ratio.

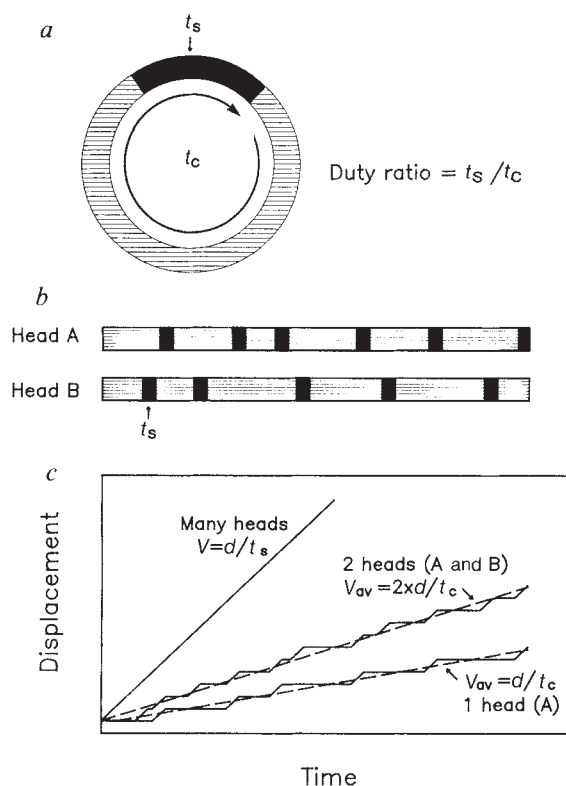


FIG. 3 Model for the origin of quantized velocities of actin filaments interacting with a small number of myosin heads. *a*, The ATPase cycle of a myosin head. The closed area indicates the duration of the strongly bound state (t_s), during which the head undergoes a stroke of step size d . The remainder of the time (hatched area), the myosin head is only weakly associated with an actin filament. The duty ratio is given by the ratio of t_s to the total ATPase cycle time (t_c). *b*, Consecutive ATPase cycles of two myosin heads A and B. Each head operates independently and stochastically. *c*, Resultant displacement of actin filaments interacting with head A or A and B, on the same time scale as in *b*. The dashed lines show the average velocity (v_{av}). The average velocity of a filament interacting with only one head is limited by d/t_c , as it makes a displacement of d at an average frequency of $1/t_c$. The average velocity of a filament interacting with two heads is limited by $2 \times d/t_c$ since it makes the same displacement at a twice higher average frequency, provided that the strokes of two heads rarely overlap with each other. For a filament interacting with an infinite number of heads, the average velocity is limited by d/t_s , which is the average speed during the period of t_s . Here, single-headed myosin subfragments are used for the sake of simplicity of explanation. In the actual experiment, we used double-headed HMM, and therefore v_u as used in the text is limited by $2 \times d/t_c$, rather than d/t_c .

Comparison with kinesin

Howard *et al.*¹⁶ and Block *et al.*¹⁷ performed similar analyses on brain kinesin and found that a single kinesin molecule was sufficient to move microtubules as fast as many kinesin molecules. This difference between kinesin and skeletal muscle myosin can be explained by the duty ratio of kinesin being much larger (close to 1) than that of skeletal muscle myosin. In other words, unlike a skeletal muscle myosin head, a kinesin head probably spends most of its ATPase cycle time strongly bound to its filament track. This hypothesis is consistent with the findings that continuous movement of actin filaments over sparse HMM surfaces depends on viscous support (methylcellulose)⁹ whereas the movement of microtubules by a single kinesin molecule does not¹⁶.

This difference in the duty cycle may reflect a division in the physiological roles of brain kinesin and skeletal muscle myosin. *In vivo*, skeletal muscle myosin works in the form of a thick filament, which contains many heads. Maintaining tension on an actin filament does not require that individual heads remain strongly bound for a large part of each cycle. By contrast, brain kinesin presumably attaches to and moves cytoplasmic vesicles that are too small to accommodate many motors. The duty ratio for this motor must therefore be quite large or the vesicle would detach and diffuse away at a very high rate. In addition, a small duty ratio allows muscle contraction to occur at much higher velocities.

Estimation of the step size

To estimate the step size from the unitary velocity, it is necessary to determine the rate at which the unit displacements occur. This corresponds in a tightly coupled system to the rate of the actin-activated ATP hydrolysis by the myosin head. It has not been possible to measure directly the cycle time of actin-activated Mg-ATPase (t_c) of HMM attached to glass surfaces at very low densities. Assuming that t_c per head under the present condition is the same as that in solution, 32 ms (which is similar to the value estimated from high density HMM-coated surfaces⁹), the step size is related to the unitary velocity of movement generated by a single HMM molecule ($v_u = 0.33 \mu\text{m s}^{-1}$) by $d = (v_u/2) \times t_c$. Both heads of HMM are assumed to be active and acting independently. Therefore v_u is divided by 2 to match t_c which is expressed in units of seconds per head, and $d \approx 5 \text{ nm}$ per ATP.

This value is remarkably small and well within constraints imposed by the size of the myosin head on the magnitude of conformational change during one power stroke, and therefore consistent with the swinging crossbridge theory. Recent crystallographic evidence from adenylate kinase¹⁸ indicates that the binding of ATP can cause a domain shift in a protein which is consistent in magnitude with that required for a 5 nm step size.

It should be noted, however, that this calculation may represent a lower limit for the step size. If only one of the two heads of HMM are active the step size would double. Second, we previously noted that the local displacement of an actin filament may not be represented in the movement of the whole filament⁹. Actin filaments are flexible and some part of the local displacement produced by interaction with one head may be dissipated in buckling or straightening of the filament. If so, the observed sliding velocity of an actin filament pushed by one HMM at its tail end would be slower than that of a filament pulled by one HMM at its leading end. Were this the case, the ideal unitary velocity, characteristic of the sliding of an actin filament pulled by one HMM at its leading end, would be greater than the apparent unitary velocity obtained from movements of filaments interacting with HMM molecules at random positions along its length. But if symmetry of the buckling/straightening behaviour is assumed, the displacement stored in a buckle will, on release, distribute itself on average equally in both directions. Therefore, even in the worst case, the ideal unitary velocity must be less than twice the apparent unitary velocity and the step

size must be, at most, doubled again. Third, even though lateral diffusion of actin filaments is suppressed by methylcellulose in this experiment, actin filaments may briefly diffuse away and stay out of reach of myosin heads. The presence of such 'dead time' will make the turnover rate of myosin heads on sparsely coated surfaces slower than their maximum rate in solution. As the effects of slower turnover rate and filament flexibility are stochastic, they should widen peaks of velocity distributions as well as displace them towards lower values. The fact that the peaks are apparent at all indicates that these effects cannot be large. Only in the case where all these inaccuracies are compounded does the actual step size approach the geometric constraint of 40 nm imposed by the size of a myosin head.

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Future prospects

Myosins from different types of cells have remarkably different maximum sliding velocities¹⁹. The method described here to observe motile activity of a single rabbit skeletal muscle HMM can be applied to characterize other forms or types of myosins. Such studies should help elucidate the functional differences between different myosins. Furthermore, this assay system provides the foundation for the analyses of dynamic properties of single myosin heads. Such analyses should be of critical importance in answering the elusive fundamental question: how do motor proteins transform chemical energy of ATP to mechanical movement? □

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A planet orbiting the neutron star PSR1829-10

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CONVENTIONAL optical techniques for detecting companions to stars have been unable to confirm the existence of other planetary systems. This is because of the small angular separation (less than an arcsecond) and relative luminosity ($\sim 10^{-10}$) of any planet with respect to its parent star. As the velocity of the star due to the motion of a planet is likely to be only about one metre per second, detection through the Doppler shift of spectral lines in the stellar atmosphere is also impractical. Here we report observations which imply the existence of a planet-sized companion orbiting a neutron star, the pulsar PSR1829-10, whose motion can be seen by Doppler effects on the observed arrival times of the pulses from the rotating neutron star. The planet is about 10 times the mass of the Earth, and is in an almost circular six-month orbit. It is not clear whether it formed in the aftermath of the supernova that created the neutron star, or was pre-existing and somehow survived through the late phases of stellar evolution and neutron-star formation. In either case, the existence of the planet challenges conventional theories of the formation of neutron stars from supernovae and has important implications for the existence of planetary systems around other stars.

The radio pulsar PSR1829-10 was discovered in 1985 in a survey of the northern galactic plane at Jodrell Bank¹, and is estimated to be ~ 10 kpc from the Sun. Since then, timing observations have been made regularly on the 40 new pulsars detected in the survey in order to determine their positions and spin-down parameters. For 39 of these, solutions have been obtained, and the results have been published elsewhere²⁻⁴. The fortieth, PSR1829-10, showed period fluctuations whose nature has only just been established.

Observations were carried out with the 76-m Lovell telescope at Jodrell Bank at frequencies between 1,400 and 1,660 MHz. Arrival times were obtained by convolution of the pulses with

an appropriate template and then corrected for the Earth's motion in the usual way, using the JPL DE200 barycentric ephemeris⁵. After fitting a standard slow-down model to the data⁶, it is clear that the residuals shown in Fig. 1a are oscillatory, with a period of ~ 6 months. The most straightforward interpretation is in terms of binary motion. We have considered other possible causes of the sinusoidal variation such as the free precession of a neutron star^{7,8} or Tkachenko oscillations in the superfluid interior of the neutron star^{9,10}. Neither of these effects has previously been identified, although the Crab pulsar shows timing noise which occasionally has a quasi-periodic behaviour for two or three cycles¹¹. However, there is no stable frequency

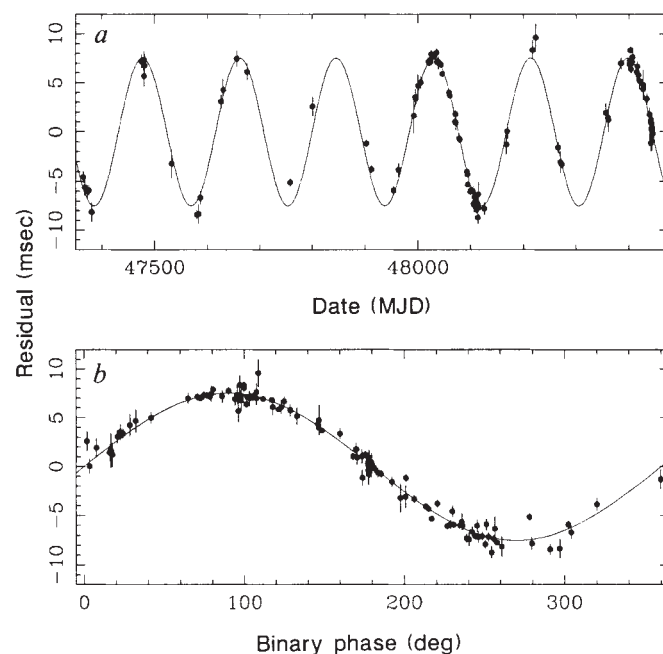


FIG. 1 The timing residuals for PSR1829-10. *a*, Plotted relative to a simple model of the slow down. The smooth curve represents the solution for a binary system with the parameters given in Table 1. *b*, As *a*, but plotted against orbital phase.

or amplitude. Although we cannot discount the possibility that PSR1829–10 shows a more pure form of this phenomenon it seems unlikely that it would be confined to this one, much older and otherwise ordinary pulsar.

Fitting for a binary motion resulted in the smooth curve shown in Fig. 1a and the parameters that are given in Table 1. As can be seen from Fig. 1, the binary model provides an excellent fit to the data. Figure 1b shows the residuals plotted as a function of binary phase. The orbit is surprisingly circular, with an eccentricity of less than 0.1. The orbital period of the planet is close to 6 months, and we were of course concerned that the modulation of the pulsar period that we are observing is not some artefact of our data acquisition or reduction techniques. Fortunately we have observations of several hundred other pulsars that have been observed and reduced with exactly the same software as PSR1829–10, and none shows a similar periodicity. In particular, the sinusoid is absent in the residuals of the pulsar PSR1829–08, which is only 2 degrees away.

The mass function, which is a property of only two observables, the projected semimajor axis $a_p \sin i$ and the orbital period P_b , gives some information about the masses of the two objects:

$$f(m_p, m_c) = \frac{4\pi^2 (a_p \sin i)^3}{G P_b^2} = \frac{(m_c \sin i)^3}{(m_p + m_c)^2} = 1.4 \times 10^{-14} M_\odot \quad (1)$$

where i is the inclination of the orbit to the plane of the sky, and m_p and m_c are the masses of the pulsar and companion respectively. Assuming that the mass of the pulsar is $1.4 M_\odot$, then the mass of the companion body is clearly very much less, and in this approximation

$$m_c \sin i = 3.0 \times 10^{-5} M_\odot = 10.2 M_E \quad (2)$$

where M_E is the mass of the Earth. For random orientation of the orbital plane, the probability that the inclination is less than some value i_0 is $1 - \cos i_0$. There is therefore a 50% chance that the mass of the companion is less than $12 M_E$ and only a probability of 0.0005 that the companion is greater than the mass of Jupiter, $300 M_E$. For any reasonable inclination then, the companion is of planetary mass.

The semimajor axis of the orbit a is readily determined from the orbital period and the mass of the neutron star:

$$a = \left[\frac{G(m_p + m_c)P_b^2}{4\pi^2} \right]^{1/3} = 1.06 \times 10^8 \text{ km} = 0.710 \text{ AU} \quad (3)$$

or about the size of the orbit of Venus around the Sun.

The measured period derivative of the pulsar gives a characteristic age of ~ 1.25 Myr, indicating that the pulsar is quite young. This is generally accepted as being an upper limit to the age of the pulsar⁶. The pulsar is losing rotational energy at a rate of $\dot{E} = I\omega\dot{\omega}$, where ω is the angular velocity of the pulsar. Assuming that the energy is radiated isotropically, and using the measured period and period derivative, and the canonical neutron star moment of inertia⁶, I , of 10^{45} g cm^2 , we estimate the energy flux at 0.7 AU to be $\sim 3 \text{ kW m}^{-2}$, which is rather similar to the solar flux incident on the Earth.

The value of $\dot{P}$ given in Table 1 is large for a pulsar with a characteristic age of 1.25 Myr. Such large values are occasionally seen in young pulsars and are usually attributed to timing noise or glitches¹². It is appealing to consider the possibility that a further, more massive, longer-period planet, perhaps like Jupiter or Saturn, is present in the system. Michel¹³ has pointed out that application of the conventional slow-down model over a limited timespan makes Jupiter-like planets difficult to detect. The presence of a Jupiter-like planet could indeed explain the observed $\dot{P}$.

In summary, there is strong evidence that we have observed a planet of mass $10 M_E / \sin i$ in a six-month circular orbit with a radius of 0.7 AU. It is either a single planet orbiting a young neutron star or, alternatively, the planet is one of a system of

TABLE 1 Measured parameters of the PSR1829–10 system

Epoch (MJD)*	48000.3
Pulsar period	330353.559543 $\pm$ 0.000008 μ s
Pulsar period derivative	4.2056 $\pm$ 0.0002(10 ^{–15})
Pulsar period second derivative	(0.28 $\pm$ 0.03) $\times$ 10 ^{–24} s ^{–1}
Dispersion measure	475 $\pm$ 1 cm ^{–3} pc
Right ascension (1950.0)	18 h 29 min 55.01 s $\pm$ 0.01 s
Declination (1950.0)	–10° 23' 50.6" $\pm$ 0.5"
Projected semimajor axis, $a_p \sin i$	7.6 $\pm$ 0.3 light ms
Orbital period, P_b	184.4 $\pm$ 0.9 days
Eccentricity, e	$\leq$ 0.1
Assumed longitude of periastron, ω	0.0°
Time of periastron (MJD)*, T_0	48168.0 $\pm$ 1.2 days
NS projected orbital velocity	0.90 $\pm$ 0.04 m s ^{–1}
Planet projected orbital velocity	42 $\pm$ 2 km s ^{–1}
Mass function, $f(m_p, m_c)$	(1.4 $\pm$ 0.2) $\times$ 10 ^{–14} $M_\odot$

* Mean Julian day.

two or more planets that may be influencing the observed value of $\dot{P}$ and $\ddot{P}$; it should be possible to establish which one of these is correct within a few years.

The existence of such a small mass in an almost circular orbit around a pulsar has implications for our understanding of the birth of pulsars, and their connection with supernovae, not to mention the presence of other planets outside the Solar System. The crucial question is whether the companion we have detected was a planet before the formation of the neutron star.

If so, then the standard theory¹⁴ of neutron-star formation poses several problems. It is widely believed that neutron stars are formed in the collapse of the cores of massive stars with an accompanying supernova explosion in which the envelope of the pre-supernova star is ejected with a large velocity of $\sim 5,000 \text{ km s}^{-1}$. The planet could therefore be destroyed or its orbit disrupted during either the pre-supernova giant phase of the star, the supernova explosion itself or afterwards. To obtain the PSR1829–10 system, the planet has had to survive all of the above and remain intact, bound and in a remarkably circular orbit.

An initial stellar mass of between ~ 6 and $60 M_\odot$ is thought to be required to create a neutron star¹⁴. Such stars are much more luminous than the Sun, but incapable of driving all but the lighter elements from the planet's surface. During the giant phase, the planet would spiral into the centre of the star if the density of the stellar atmosphere at the distance of the planet were to become too large¹⁵. As we cannot uniquely determine the mass of the pulsar progenitor, and hence the radius of the giant, it is possible that the planet would survive this phase.

The supernova explosion, however, poses a serious threat to the survival of a bound and intact planet. Even if the explosion were symmetric, then the system would only remain bound if the amount of matter ejected from the system were less than half of the total mass¹⁶. Furthermore, there seems to be good evidence that radio pulsars also receive a velocity impulse due to an asymmetry in the explosion of ~ 100 – 200 km s^{-1} (refs 17, 18). For small kick velocities of $\sim 100 \text{ km s}^{-1}$, the survival probability of the binary is fair, ~ 0.1 , whereas the probability that an almost circular orbit would result is very small indeed ($\leq 10^{-4}$). A further disrupting effect will be caused by the impact of the outgoing supernova shell on the planet. This will impart a velocity impulse which depends on the fraction of the shell intercepted, and on its momentum¹⁹. For $3 M_\odot$ of ejecta and a planet of the same density as the Earth and ten times the mass, then at the present distance from the pulsar, the velocity gain would amount to only $\sim 2 \text{ km s}^{-1}$. As this is small compared with the pre-supernova orbital velocity, it would have little effect on the subsequent orbit. The planet also has to survive the incident blast wave. If the energy absorbed from the impact is comparable to the binding energy of the planet, then the planet could be destroyed. For this to be the case, the incident kinetic energy must be a few orders of magnitude greater than the binding energy, because most of the incident energy is not

absorbed. For the parameters above, however, the kinetic energy of the outgoing supernova shell that would hit the planet is only ~ 10 times the binding energy, suggesting that the planet would survive the impact.

Should the planet remain intact and bound, then there is the possibility that radiation from the pulsar would destroy it. But it has been suggested¹⁵ that a planet of $5 M_E$ could survive the presence of a Crab-like pulsar at a distance of 0.33 AU. We have no reason to expect that PSR1829–10 was any more energetic than the Crab pulsar, and so it seems that the planet could have survived the incident radiation.

The chief problem is the low eccentricity of the orbit, given the effects of the supernova described above²⁰. Tidal circularization of an orbit is only efficient when the radius of one or more of the bodies is similar to the radius of the orbit. For both the neutron star and the planet, this seems extremely unlikely. A radially driven wind from the planet may help to circularize the orbit if the ratio of the specific energy to angular momentum of the outgoing material were greater than that of the planet. Therefore, if the pulsar removed a significant fraction of the mass of the planet, it may be possible that in the process the planet's orbit could become circular.

Clearly, there are difficulties in explaining how a planet could have survived the giant phase of evolution of its companion, the supernova explosion and been retained in a circular orbit. Even if the collapse to a neutron star produces no ejecta other than the mass equivalent of the binding energy, which is mostly ejected in the form of neutrinos, this process would introduce an eccentricity of ~ 0.1 depending on the equation of state of the neutron star.

Another possibility is that the planet was formed after the neutron star from a keplerian disk. Such a disk may have originated from the complete tidal disruption of a companion star, or fall-back of material following the supernova explosion. A further possibility (M. Ruderman, personal communication) is that the pre-collapse stellar core is spinning too fast to collapse to a neutron star while conserving angular momentum, resulting in a slower collapse and the formation of a disk. Whether or not in any of these cases the disk can extend to a distance of 0.7 AU, the current distance of the planet, is unclear, but such a process would naturally explain the circularity of the orbit.

On the other hand, the planet could once have been a companion star which has been reduced to its current mass by some mechanism. One possibility is that the system was once a binary, and radiation from the pulsar has been annihilating the companion²¹. The second is that the pulsar has been residing inside the envelope of the companion and ejected most of the mass²². Although both of these scenarios make an explanation of the circular orbit much easier, the present mass of the planet is more difficult to explain. It seems unlikely that the process responsible for removing most of the mass of the star would cease when the star is reduced to $\sim 10^{-4}$ of its original mass. The alternative, that the process is continuing and that we are witnessing the last few Earth masses being annihilated is also unlikely given the modest energy flux currently being received by the body.

The projected orbital velocity of the neutron star due to the planetary motion amounts to only 0.9 m s^{-1} . The measurement of such a velocity is only possible using radio pulsars because of their outstanding rotational stability. Earth-like planetary orbits are difficult to detect because of the small size of the perturbations in the neutron star position. Indeed, simulations show that if the Sun were a pulsar, it would be difficult to deduce the presence of a planetary system over only a 5-year timespan. We have been fortunate that the period and mass of this planet allowed its detection over a relatively short time. Observations over a longer time interval may reveal the presence of other planets in the system.

The existence of a planet in the PSR1829–10 system is not easy to explain using conventional theories of neutron-star

formation and supernovae. We may be able to reconcile ourselves to all of the improbabilities involved in the survival of a planet if we remember that, although there are over 500 known pulsars, PSR1829–10 is the only system in which one is clearly identifiable. Unless PSR1829–10 is showing some remarkable new form of rotational instability, we believe that this is the first detection of a planetary-sized body outside the Solar System. $\square$

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A disrupted radio jet inside the host galaxy of the quasar 3C48

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THE nearby quasar 3C48 was the first to be optically identified¹, and its redshift, $z = 0.368$ (ref. 2), was the second, after that of 3C273³, to be determined. Despite this pedigree, its detailed radio structure has remained obscure because its angular size, ~ 1 arcsec, is so small. We present here a new radio image, with resolution < 10 mas, made by very-long-baseline interferometry using an array of 17 telescopes. We find a highly disrupted radio jet quite different from those found in standard high-luminosity radio sources. The jet lies well within the body of the gas-rich host galaxy of the quasar, and we believe that its irregular shape is due to collision with a dense clump of gas in the interstellar medium of the host galaxy, rather than to any irregularity or precession of the central engine that produces the jet.

The quasar 3C48 was one of four sources observed during a series of very long baseline interferometer (VLBI) observations at 1.6 GHz in April 1984. Seventeen telescopes in Europe, the Soviet Union and North America contributed to the observations (see refs 4, 5 for a description of the telescopes involved and

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the initial data reduction). We self-calibrated⁶ the visibility data for 3C48 in the Jodrell Bank OLAF software package and performed the final deconvolution step in the National Radio Astronomy Observatory AIPS package using the maximum entropy deconvolution algorithm, VTESS⁷; this image is shown in a false-colour representation in Fig. 1. (Other versions of the image can be seen in ref. 8.) We also made a lower-resolution image (60 mas) from a combination of data from the Jodrell Bank MERLIN⁹ and the European baselines in our VLBI array; the result is shown as a contour map in Fig. 2. Looking first at Fig. 1, the active nucleus or core has been identified¹⁰ from its inverted radio spectrum with the relatively weak southernmost component, A, which is elongated north-south (see Fig. 1). About 50 mas to the north of A ($150 h^{-1}$ pc for $H_0 = 100 h$ km s⁻¹ Mpc⁻¹ and density parameter $q_0 = 0.5$) is a bright compact component, B, over four times as intense as A. This component is slightly resolved and is also elongated roughly north-south (see Fig. 1). As A is the nucleus, we shall call B a 'hot spot', although no spectral information is available to confirm the identification. North of B, the outline of the emission expands and extends over ~ 500 mas ($\sim 1.5 h^{-1}$ kpc). Figure 2 shows,

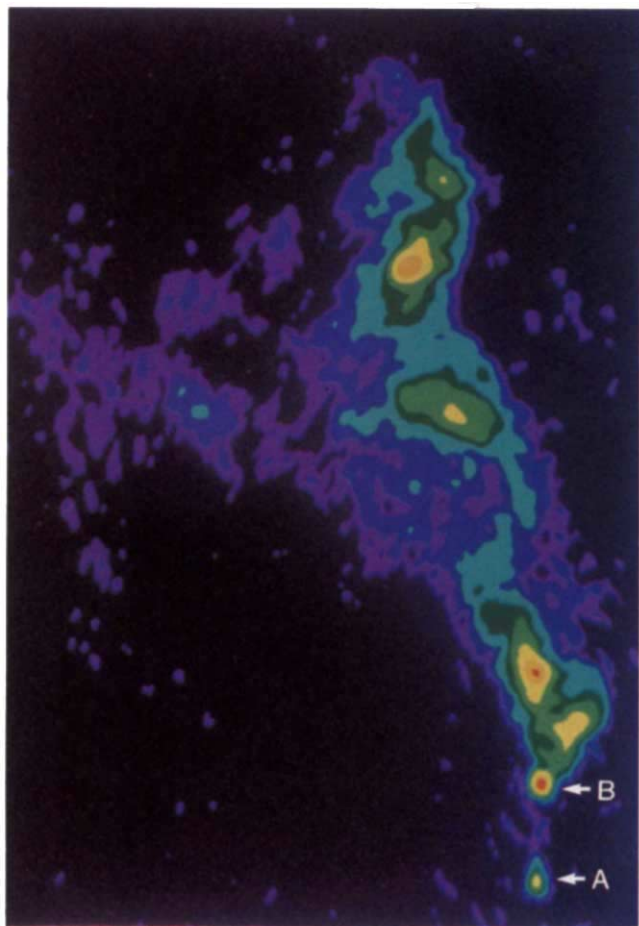


FIG. 1 False-colour image of the radio source 3C48 made from 17-station intercontinental VLBI data at 1.66 GHz. The image extends 505 mas from north (top) to south and 355 mas from east (left) to west; the resolution is 6 mas and the r.m.s. noise level off source is $\sim 500 \mu\text{Jy}$ per beam⁻¹. The active core has been identified¹⁰ with the component A at the southernmost tip whose peak brightness is 60 mJy per beam; it is elongated north-south (an elliptical gaussian fit yields 7.5×2.5 mas (FWHM) in position angle 0.5°). The intense hot spot B roughly 50 mas ($150 h^{-1}$ pc) to the north of A is slightly resolved (5.7×3.0 mas in position angle 177°); it has a peak brightness of 276 mJy per beam. The colours represent the following brightnesses (all in mJy per beam): purple > 2 ; blue > 3 ; light blue > 6 ; green > 13 ; light green > 20 ; yellow > 40 ; orange > 90 ; red > 180 .

however, that this diffuse emission actually extends out to ~ 1 arcsec ($3 h^{-1}$ kpc) from the core.

This radio structure is highly unusual. VLBI images of powerful galactic nuclear sources such as this often show an unresolved core plus a narrow jet which is usually unresolved perpendicular to its length. Powerful, extended (> 50 kpc) double sources have central compact cores and jets which terminate in hot spots at the outer edges of diffuse lobes straddling, and well outside, the parent object. Weaker extended double sources do not have outer hot spots; the oppositely pointing jets expand and fade as they propagate into the intergalactic medium. The radio source in 3C48 is buried deep within the host galaxy, and yet with its diffuse expanding jet and intense hot spot, it shows a mixture of features found in the extended double sources. Instead of being located at the outer edge of the source, however, the hot spot in 3C48 is close to the core.

It has been suggested^{11,12} that the interaction of a jet with gas inside the host galaxy may be the cause of the small sizes and unusual shapes of many compact steep spectrum sources identified with quasars. 3C48 is such a quasar whose radio structure has now been determined in more detail than any other. Our images provide powerful direct evidence that the chief determinant of its radio structure is indeed an interaction with the interstellar medium of the host galaxy rather than, for example, a variation in the jet direction due to precession of the central engine. Our model for 3C48 is that the active nucleus A produces a 'cold' beam, or jet, of energetic particles which radiates weakly. The jet then 'splashes' against a cloud at the hot spot B and its highly directed energy is partly thermalized; the relativistic particles begin to radiate freely and exert a considerable pressure. The ambient medium around B partially confines the resulting expansion and the pressure gradient in the interstellar medium helps to channel the flow in the forward direction. The jet finally comes into pressure balance with its surroundings. Apart from the appearance of our images, what evidence is there to support this model?

There is strong evidence that the radio source is embedded within a galaxy. The initial identification plate showed that the stellar object is surrounded by a faint nebosity¹. More recent images of the extended optical 'fuzz'¹³ suggest that it is associated with a spiral galaxy. To the south the 'fuzzy' light is dominated by continuum emission, largely from A-type stars, whereas to the northwest it is dominated by emission lines¹³⁻¹⁷. Two pieces of evidence then suggest that this galaxy contains much more gas than does a typical active galaxy.

First, 3C48 is one of the relatively few quasars detected by the infrared astronomy satellite IRAS, and it has a very high flux at 60 μm indicating a large 'infrared bump' in its spectrum¹⁸; the infrared luminosity, $5 \times 10^{12} L_\odot$, dominates the luminosity of the entire quasar. Neugebauer *et al.*¹⁸ interpret this enormous infrared luminosity as reradiation from dust, and, assuming a normal dust-to-gas ratio, they infer that $> 10^{10} M_\odot$ of gas is associated with this dust; the distribution of this dust and gas is, however, unclear. Second, Fabian *et al.*¹⁹ infer the presence of 3×10^8 to $2 \times 10^9 M_\odot$ of ionized gas in the extended nebosity from observations of spatially resolved optical spectra out to 30 kpc. Significantly, Fabian *et al.* also infer that 3C48 is at the centre of a massive cooling flow with an inflow rate of $\sim 100 M_\odot \text{ yr}^{-1}$.

If we accept that the jet is propagating through a gas-rich environment, what causes the disruption at B? The jet-gas interaction must be much stronger than is typically the case in massive early-type galaxies, some of which are close enough for their X-ray, optical and radio properties to have been studied in some detail²⁰. It has been hypothesized^{20,21} that the weakest jets found in these early-type galaxies are disrupted by a shock induced as the jet propagates through the 'pressure wall' at the sonic radius of an X-ray-emitting cooling flow. This could explain why the weakest of these radio sources (whose power at 5 GHz is less than $10^{22} \text{ W Hz}^{-1}$) lie within the central

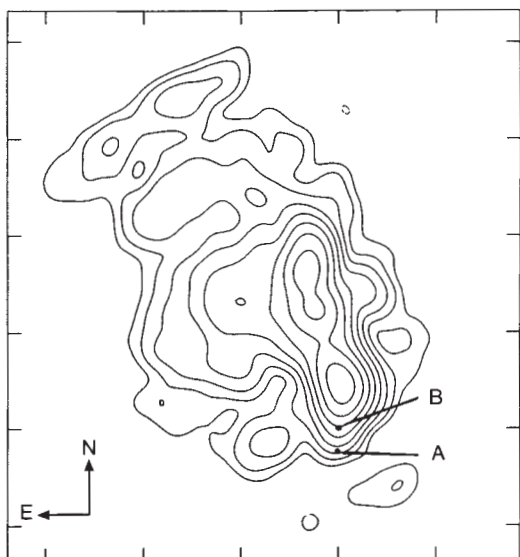


FIG. 2 Image of 3C48 made from a combination of MERLIN and European VLBI data at 1.66 GHz; the resolution is 60 mas. North is at the top and east at the left of the image; the ticks around the border mark intervals of 0.2 arcsec. The emission extends over ~ 1 arcsec ($\sim 3 h^{-1}$ kpc) and accounts for virtually all the total flux density of 3C48 at 1.66 GHz (13.9 Jy; ref. 29). The contours are drawn at $-1, -0.5, 0.5, 1, 2, \dots, 32$, 64% of the peak brightness of 1.80 Jy per beam.

kiloparsec of the host galaxy, while the most powerful (whose power at 5 GHz is more than 10^{24} W Hz $^{-1}$) are larger than the host galaxy²⁰. 3C48 is five orders of magnitude more powerful and yet is as small as the weakest of these galactic radio sources, which suggests that a more violent disruption mechanism is at work. The fact that the source outline starts to expand at the position of an intense hot spot leads us to think that here we are witnessing a jet hitting a gas cloud in the interstellar medium of the host galaxy. The obstacle could be a cool ($\sim 10^4$ K) condensation in the cooling flow, formed at a radial distance where the hot diffuse gas becomes thermally unstable. For an inflow rate of $\sim 100 M_{\odot} \text{ yr}^{-1}$, we estimate that the density of the diffuse cooling flow near B will exceed $10^{-19} \text{ kg m}^{-3}$; depending on the metal abundance, the cooling time will be 10^5 – 10^6 years. The dynamical time for the infalling gas is somewhere in the middle of this range, and therefore the conditions for thermal instability of the flow may well be met.

The total amount of energy carried by the jet is likely to be large, $>10^{38}$ W, as the current radio luminosity of 3C48 is $\sim 4 \times 10^{37}$ W. This powerful jet will excavate a cavity in the cool cloud, and thermalized material will flow out of the cavity and into the interstellar medium. In this model, however, the jet must be striking the obstacle a glancing blow, for most of its momentum is still directed forwards. In Fig. 1 there is no sign of backflowing material between A and B, and it is backflow that forms the dominant lobes in powerful doubles after their jets have struck the intergalactic medium at a hot spot. The density gradient in a cooling flow will also help to direct the flow forwards; the disrupted jet will preferentially expand down the pressure gradient ($\propto r^{-2}$) and hence outwards from B.

Further speculation along these lines is inappropriate without more information on the physical conditions around the jet, but because of the jet's location deep within a distant galaxy this will be difficult to obtain. The Hubble Space Telescope can, however, obtain spatially resolved images of 3C48, and observations of the line-emitting gas would be particularly useful. In addition, numerical modelling of a powerful jet propagating through a dense, clumpy galactic atmosphere is needed to get a better feel for the origin of the flow patterns captured in our images.

Two further points of interpretation are, however, worth men-

tioning. The minimum pressure in the disrupted jet to the north of B is a few times 10^{-8} N m^{-2} (from synchrotron theory). The expansion of the source outline indicates that here the pressure in the radio-emitting plasma exceeds that of the surrounding gas. In the outer, relaxed, parts of the radio source seen in Fig. 2, the minimum pressure in the radio-emitting plasma is $\sim 10^{-9} \text{ N m}^{-2}$. Extrapolating the run of pressure in the cooling flow model of Fabian *et al.* (by more than an order of magnitude) yields an estimate for the pressure in the interstellar medium ~ 1 kpc from the flow centre of $\sim 10^{-9} \text{ N m}^{-2}$. The outer part of the jet has, therefore, probably come into pressure equilibrium with the interstellar medium. The total energy in the radio plasma ($>10^{51}$ J) dumped into the narrow-line-emitting region a few kiloparsec from the nucleus corresponds to the kinetic energy of $10^9 M_{\odot}$ of gas moving at $1,000 \text{ km s}^{-1}$. Thus the expanding radio source can affect the dynamics^{22–24} of even this massive line-emitting region. It is therefore noteworthy that the line width of [OIII] in 3C48, $\sim 1,200 \text{ km s}^{-1}$ (N. Jackson, personal communication), is significantly larger than usual in active galactic nuclei; this may in part be due to the interaction between the radio source and the narrow-line-emitting region.

Finally, our images show only one jet in 3C48; the brightness ratio on opposite sides of the core is more than 100:1. Asymmetries in powerful radio sources are often ascribed to the effects of relativistic aberration ('Doppler beaming') on an intrinsically two-sided source²⁵ whose axis lies close to the line of sight. But it is hard to sustain this argument in this case. The core of 3C48 is very weak ($\sim 0.4\%$ of the total flux density at 1.6 GHz) for a radio-loud quasar, and on the basis of the 'unified scheme'^{25,26} this implies that the jet axis is close to the plane of the sky. The simplest conclusion to draw from our observations is that radio emission from 3C48 is intrinsically one-sided. Such a powerful one-sided jet makes 3C48 a candidate to be a 'galactic rocket'^{27,28}. Using the Hubble Space Telescope and radio interferometry, it may be possible to compare the position of the radio core with the optical centre of the galaxy to an accuracy of better than 10 mas ($<30 h^{-1}$ pc). High-thrust rockets can move further from the bottom of the galactic gravitational potential well than this²⁸. □

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Carbon suboxide in comet Halley?

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OBSERVATIONAL data acquired during the recent appearance of comet Halley pose a puzzle about the nature and distribution of elemental carbon and carbonaceous material in its nucleus and coma. The nucleus is darker even than coal (albedo < 4%)¹, suggesting that its volatile ices contain a few per cent of carbonaceous material in the form of graphitic or amorphous carbon. The very high abundance of light elements in the coma dust^{2,3}, particularly H, C, N and O, suggests the presence of a significant organic component. The emission feature near 3.4 μm also implies the presence of organic material in the dust⁴⁻⁶. But the parent species for the primary carbon-containing material that have been identified so far (such as CO, CO₂ and CH₄) are not present in sufficient quantities to account for all of it. Here we propose that an additional contribution from carbon suboxide (C₃O₂) in the coma dust and the nucleus material is consistent with the observational data. A production rate in the coma for C₃O₂ of about 0.03–0.04 times that of water would provide the distributed source of elemental carbon and CO within 10⁴ km of the nucleus that is required to explain the data from the Giotto spacecraft and from ground-based observations.

Consistent with the high carbon content of nucleus and dust material, there are considerable observations of carbon compounds in the coma gas. Eberhardt *et al.*⁷ interpret the spatial distribution of the signal at mass/charge ratio $m/q = 28$ AMU/ e obtained by the neutral-gas mass spectrometer (NMS) on the Giotto spacecraft to derive a carbon monoxide production rate, Q_{CO} , which was 7% that of the water production rate, $Q_{\text{H}_2\text{O}}$, near the nucleus, and 15% of $Q_{\text{H}_2\text{O}}$ at a distance of $R \approx 2 \times 10^4$ km from the nucleus. The presence of an additional, extended source of gas-phase carbon monoxide in the inner coma ($R < 2 \times 10^4$ km) with a carbon monoxide production rate 8% that of water was therefore suggested. In addition, the spectrometer also detected carbon dioxide, CO₂, with $Q_{\text{CO}_2}/Q_{\text{H}_2\text{O}} \approx 0.04$ inferred^{8,9}. Methane (CH₄) in the coma gas was first identified from the Giotto ion mass spectrometer (IMS) data; a model analysis⁹ of the 'H₂O group' ion measurements inside the contact surface ($R < 5 \times 10^3$ km) yields $Q_{\text{CH}_4}/Q_{\text{H}_2\text{O}} \approx 0.02$. This experiment also detected an unexpectedly large abundance of the C⁺ ion, more than can be produced by photo-processing of the observed amounts of CO, CO₂, and CH₄ in the coma¹⁰. Balsiger *et al.*¹⁰ suggest that carbon atoms (C⁰), or some species readily photolysed to carbon atoms, might be emitted directly from the nucleus or from dust in the coma, and subsequently photolysed to yield the observed C⁺. The estimated production rate for the extra source of C⁰ (or C⁺) was 5–10% that of water.

Ground-based and Earth-orbit observations of Halley complement the Giotto measurements. Woods *et al.*^{11,12} observed spatial profiles of carbon monoxide as well as the 1,657 Å emission of atomic carbon during sounding rocket flights. Using a simple radial outflow (Haser) model, they determined a production rate for carbon monoxide 17–20% that of water (similar to the value derived from International Ultraviolet Explorer satellite spectra¹³). Adopting a coma composition including CO, CO₂ and CH₄ with production rates of 20%, 3.5% and 7% of the water production, respectively, they found that the C⁰

fluorescence that would result from this model is about an order of magnitude less than that observed for $R < 10^5$ km. They postulated¹¹ additional production of carbon at a rate 3% of the water production rate, either at the nucleus or in the inner coma ($R \leq 10^4$ km), to fit the observations. They also suggested¹² that adding to their model electron-impact ionization of CO (with a frequency $4.1 \times 10^{-6} \text{ s}^{-1}$) followed by dissociative recombination of its ion CO⁺ (with a frequency $3.8 \times 10^{-4} \text{ s}^{-1}$) would yield a good fit to the observed spatial distribution of 1,657 Å emission from carbon. The extra carbon production inferred from these observations may even be larger, as the model overestimated the CO and CH₄ production rates (in comparison with the *in situ* Giotto measurements); this would then be consistent with the enhanced carbon production rate inferred from the Giotto IMS data¹⁰.

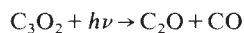
The electron-impact source of C⁰ proposed by Woods *et al.*¹² was based on data from the Vega 2 electron spectrometer (PLAS-MAG-1)¹⁴ and the work of Cravens *et al.*¹⁵ The electron-impact ionization frequency for CO in the inner coma can also be calculated from results produced by the three-dimensional electron spectrometer of the Giotto RPA-Copernic experiment¹⁶, which obtained data closer to the Halley nucleus than was possible from the Vega 2 flyby. Giotto results for the electron flux for $R \approx 1.5 \times 10^4$ km have been obtained for energies above ~20 eV (D. Larson and R. Lin, personal communication); the electron flux decreases monotonically out to ~1 keV (above which the background count rate dominates the instrumental signal). At electron energies less than 600 eV, the Giotto results are systematically smaller than the Vega data by a factor of ~6. In addition, the peak in the Vega results near 1 keV is not present in the Giotto data nor in any other solar-wind electron spectrum. This suggests that there may be a problem with the PLAS-MAG-1 instrument, the possibility of which was discussed by Gringauz *et al.*¹⁴. The multidirectional viewing capability of the RPA-Copernic experiment permits correction for signals due to dust impact and secondary electrons from neutral gas striking the detectors. From published CO cross-sections¹⁷ and the Giotto electron fluxes (including an estimate for electron fluxes near 10 eV), an electron-impact ionization frequency for CO of $\leq 1 \times 10^{-7} \text{ s}^{-1}$ for $R \leq 2 \times 10^4$ km is calculated (D. Larson and R. Lin, personal communication). As the ionization due to the probably spurious 1-keV peak in the Vega electron-flux measurements contributes half of the calculated impact ionization of CO (ref. 12, 15), elimination of this contribution and reduction of the remaining PLAS-MAG-1 spectrum by a factor of 6 yields an ionization frequency consistent with the calculation using the Giotto data. As the Giotto-based ionization frequency for CO is less than one-tenth of the value used by Woods *et al.*¹², the previously proposed electron-impact ionization mechanism may not be important for generating the extra carbon atoms observed. We pursue instead the suggestion by Woods *et al.*¹¹ that an unidentified carbon-bearing molecule with a short lifetime ($\leq 10^4$ s) could be the source of the extra carbon.

One short-lived parent species that could furnish the distributed source of CO is formaldehyde (H₂CO), unambiguously detected in comet Halley by its 6-cm radio emission line¹⁸. The deduced production rate for H₂CO was 1.5% that of H₂O (only part of the 8% required by the Giotto NMS data). Polymerized formaldehyde ($-\text{H}_2\text{CO}-$)_n (polyoxymethylene, or POM) has been suggested as a component of the coma dust¹⁹⁻²¹ and its decomposition could provide the 'extra' CO and the observed H₂CO (refs 22, 23). However, the laboratory measurements for POM necessary for photochemical models of the Halley coma are not available. Although decomposition of coma POM could be the distributed source of CO, it seems that it also cannot provide the extra measured C⁰, as we have shown above that electron impact on CO, proposed as a source of C⁰ (ref. 12), is not adequate to match the observations.

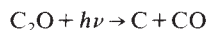
An intriguing aspect of the Halley observations of C⁺, C⁰ and CO is that previously unidentified sources are invoked in all

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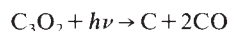
analyses. We propose that a common solution exists: the presence of carbon suboxide (C_3O_2) in the coma dust (and nucleus material)²⁴. Carbon suboxide is a well-characterized oxide of carbon composed of a carbon atom bonded to two carbon monoxide groups in a linear structure ($O=C=C=O$). Pure C_3O_2 is a stable liquid between 166 and 280 K (ref. 25). At cometary temperatures, C_3O_2 is stable in the condensed phase, and its vapour pressure²⁵ is many orders of magnitude smaller than that of CO. In the gas phase, C_3O_2 dissociates at the C–CO bond upon absorption of ultraviolet light of wavelength $\lambda < 450$ nm (refs 26, 27),



In turn, C_2O photodissociates rapidly at $\lambda < 380$ nm (ref. 27)



In the vacuum ultraviolet ($\lambda < 206$ nm), photolysis of C_3O_2 leads directly to the scission of both C–CO bonds^{27–30},



Photodissociation of C_3O_2 is used commonly in the laboratory as a source of atomic carbon.

Either thermally (above 290 K) or by ultraviolet photolysis, C_3O_2 is easily converted to two CO molecules and one carbon atom. Therefore decomposition of C_3O_2 on dust grains or in the coma gas produces CO and C^0 in a 2:1 ratio which compares well with the relative production rates for the extra (extended) sources of CO ($\sim 8\%$ relative to H_2O) and C^0 ($\sim 3\text{--}5\%$ relative to H_2O) indicated by the Halley observations. To fit these observations quantitatively, an integrated production rate for C_3O_2 of 3–4% relative to that of H_2O is required. Carbon suboxide is an alternative to POM as the source of the extra CO and, uniquely, provides a source of C^0 .

Using published photoabsorption cross-sections^{26,31} and a solar flux appropriate for the day on which Giotto flew by Halley⁹, we calculate a total C_3O_2 photolysis frequency of $5.32 \times 10^{-4} \text{ s}^{-1}$. This yields a characteristic scale length of 1,878 km for a typical outflow velocity of 1 km s^{-1} near $R = 10^4$ km (ref. 32). If the peak release of C_3O_2 from coma gains and subsequent rapid photodecomposition occur at $R \approx 8\text{--}10 \times 10^3$ km, the short C_3O_2 scale length yields a spatial distribution for CO in agreement with the extended source of CO measured by the Giotto NMS instrument⁷ and also indicates that peak C^0 production occurs for $R \leq 10^4$ km. The latter is what is required for the additional carbon source to match the observed C^0 emission¹¹, further supporting the hypothesis that C_3O_2 is a common source for the extra C^0 and CO observed in Halley. Carbon suboxide also may vaporize directly from the nucleus and, because of its short scale length, could be an important source of CO detected near the nucleus by the Giotto NMS.

Two different evolutionary histories could explain the proposed presence of C_3O_2 in cometary nuclear ices. Carbon monoxide is the second most abundant detected molecule (after H_2) in interstellar molecular clouds³³. If cometary ices are an agglomeration of material from the interstellar cloud out of which the Solar System formed³⁴, Halley's proto-nucleus would consist of a large fraction of native CO. In laboratory experiments, upon exposure to high-energy radiation (photons or charged particles), C_3O_2 is formed from CO in the gas phase^{35–37}, liquid³⁸ or condensed phase^{39–41}. The yield of product C_3O_2 depends on the purity of the CO sample³⁷. During its lifetime, mostly spent in the 'Oort cloud' at the outer reaches of the Solar System, the Halley nucleus has been bombarded by cosmic rays, and during its passages through the inner Solar System, irradiated by the solar ultraviolet radiation field. Presumably, some fraction of the primordial CO would have been converted to C_3O_2 , the amount being a function of the homogeneity of the nuclear volatiles. If domains rich in CO were present, a large quantity of C_3O_2 may have been created over time.

Alternatively, a fraction of the unprocessed interstellar

material accumulated in the Halley proto-nucleus may have been C_3O_2 . The presence of C_3O_2 in interstellar molecular clouds is difficult to ascertain because C_3O_2 is a symmetric, linear molecule and therefore does not possess a permanent dipole moment. Consequently, it does not have strong transitions at wavelengths greater than $350 \mu\text{m}$, the spectral range in which molecular cloud constituents are usually detected. But the species C_3O , structurally similar to C_3O_2 , and the sulphur analogues C_3S and C_3S have been detected in interstellar molecular clouds³³, indicating that the interstellar existence of C_3O_2 is plausible.

Carbon suboxide, or other members of the homologous series $C_n(\text{CO})_2$ (where $n = 1, 2, 3 \dots$) which might also be present in ices in the comet nucleus or dust, may be the source of other carbon species observed in the Halley coma gas. In laboratory experiments⁴², electron bombardment of adsorbed C_3O_2 results in its decomposition to form C_3 . A similar process occurring in the comet might be the source of C_3 species which have been observed in the coma gas, but whose parent species has never been convincingly identified⁴³. Alternatively, C_5O_2 may be expected to decompose to yield $C_3 + 2CO$. The observed diatomic carbon C_2 (ref. 43) may be the product of C_4O_2 decomposition.

Further evidence for C_3O_2 in the coma gas may be sought in the final calibrated Giotto NMS and IMS data. The parent peak of C_3O_2 in mass spectral tables is C_2O^+ ($m/q = 40$)⁴⁴. Although other chemical species have the same mass (such as NaOH, MgO, Ar, Ca and C_3H_4), the variation of signal intensity at $m/q = 40$ with distance from the nucleus should allow us to identify the presence of C_3O_2 . Laboratory experiments involving the irradiation of mixtures of ices that are likely candidates for the composition of cometary nuclei, as well as experiments to assess the chemical stability of C_3O_2 in a H_2O ice matrix, would help to constrain the proposed presence of C_3O_2 in the Halley nucleus. Detection of C_3O_2 in interstellar clouds by highly sensitive infrared observations would support a primordial origin for cometary C_3O_2 . $\square$

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Observation of synchronous picosecond sonoluminescence

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SONOLUMINESCENCE^{1–13} is a non-equilibrium phenomenon in which the energy in a sound wave becomes highly concentrated so as to generate flashes of light in a liquid. We show here that these flashes, which comprise over 10^5 photons, are too fast to be resolved by the fastest photomultiplier tubes available. Furthermore, when sonoluminescence is driven by a resonant sound field, the bursts can occur in a continuously repeating, regular fashion. These precise 'clock-like' emissions can continue for hours at drive frequencies ranging from audible to ultrasonic. These bursts represent an amplification of energy by eleven orders of magnitude.

In a resonant sound field, sonoluminescence (SL) originates from the pressure antinode in a region less than $10\ \mu\text{m}$ in diameter and comes in repetitive bursts. Our measurements indicate that each of these bursts has a power greater than $1.0\ \text{mW}$ and a duration less than $100\ \text{ps}$. By comparison, the fastest visible transition ($3 \rightarrow 2$) of the hydrogen atom is over 100 times slower. The repetition rate for these bursts is the frequency, f_a , of the sound field. Typically, $f_a = 10^4$ cycles per second, which corresponds to an acoustic period of $100\ \mu\text{s}$, so that the pulse width Δt_{SL} is over 10^6 times smaller than the period of the sound field. A third important timescale is the wander in the difference in phase of the acoustic cycle at which consecutive bursts are emitted, $\Delta\tau_{\text{SL}}$, which can be less than $200\ \text{ps}$. The light emission is visible to the unaided eye and appears blue. We therefore estimate its frequency, E_{photon}/h , to be $8.0 \times 10^{14}\ \text{Hz}$. This phenomenon is therefore characterized by a broad spectrum of times

$$E_{\text{photon}}/h \gg 1/\Delta t_{\text{SL}} \gg 1/\Delta\tau_{\text{SL}} \gg f_a \gg 1/\tau_M \quad (1)$$

where τ_M (modulation time) is the timescale, typically $\sim 1\ \text{s}$, on which the intensity blinks on and off in certain regions of parameter space (regions of pressure and frequency).

Sonoluminescence was discovered over 50 years ago. In early studies of the effects of high-intensity sound fields on chemical reactions⁵, it was found that H_2O_2 was formed in water in the presence of cavitating bubbles⁶. The quantum of energy needed to create peroxide from water is extremely large compared with the sound-field energies⁷, and Mecke suggested that these quanta were sufficient to cause emission of visible light. This prediction was borne out by experiments⁴ that refer to Mecke's otherwise unpublished work.

In the standard model for sonoluminescence^{1,3}, collapse of a bubble formed by cavitation occurs in a sufficiently spherical and adiabatic manner that the energy of collapse is delivered to a small number of molecules, which are thus excited or dissociated to the point at which they emit light (as chemiluminescence) when they recombine. The distribution, location and timing of cavitation have been largely unpredictable. Saksena and Nyborg⁸ have, however, observed SL in the absence of cavitation noise, and Crum and Reynolds⁹ have imaged a distribution of bubbles that also produced SL without

cavitation noise. Recently Gaitan¹⁰ and Crum¹¹ showed that a single cavity could be driven hard enough to emit stable sonoluminescence. These experimental advances (along with our calculation of the energy amplification) motivated us to investigate the properties of controlled SL.

Our apparatus consists of spherical glass flasks filled with a 25% solution (by volume) of glycerine in water. A vacuum pump is used to degas the mixture, enabling us to reach the high-intensity sound field required for SL. A radially polarized, hollow cylindrical piezoelectric drive is mounted on the flask with its axis pointing to the centre of the sphere. This transducer is driven by the amplified signal from a waveform generator (HP3325A). Our investigations are limited to radially symmetric modes. A broad-band photomultiplier tube (PMT) is used to characterize the emitted pulses of light. Figure 1a shows the range of sound pressure amplitudes, at the centre of the flask, for which stable steady-state SL can be observed at different frequencies. The amplitudes were calibrated with a microphone (B&K 4138) held a few centimetres from the flask. From the radiated acoustic field, and the transmission coefficient, we determined the field in the fluid.

The phenomenon of SL involves an extraordinary degree of energy focusing. A typical SL sound field has a pressure swing p' of $\sim 1\ \text{atm}$, which in water corresponds to a Mach number M of 10^{-5} ($M = v'/u$, where v' is the speed of the fluid motion and u is the propagation speed of a sound wave). The energy density of the sound field is then $U = (1/2)\rho(v')^2 \approx 17.3\ \text{erg cm}^{-3}$ ($\approx 1.08 \times 10^{-11}\ \text{eV per atom}$) where ρ is the liquid density. As a photon must originate from a molecule, ion or atom and as a blue photon has an energy of $\sim 3\ \text{eV}$, this phenomenon involves a focusing or amplification of about eleven orders of magnitude. The size of the spontaneous energy concentration that characterizes SL is so large that one must wonder about the limits of amplification that can be achieved with this type of non-equilibrium phenomenon.

The accumulation of many bursts by a sampling oscilloscope leads to the solid curve in Fig. 2a. Note the 2.2-ns 'rise' time. Figure 2b shows a response curve obtained by using a 34-ps laser pulser as a light source. The two curves are virtually indistinguishable, suggesting that the width of the emission is substantially less than the limitations imposed by the PMT (rated at 2.2-ns rise time). Thus we attempted to determine the duration

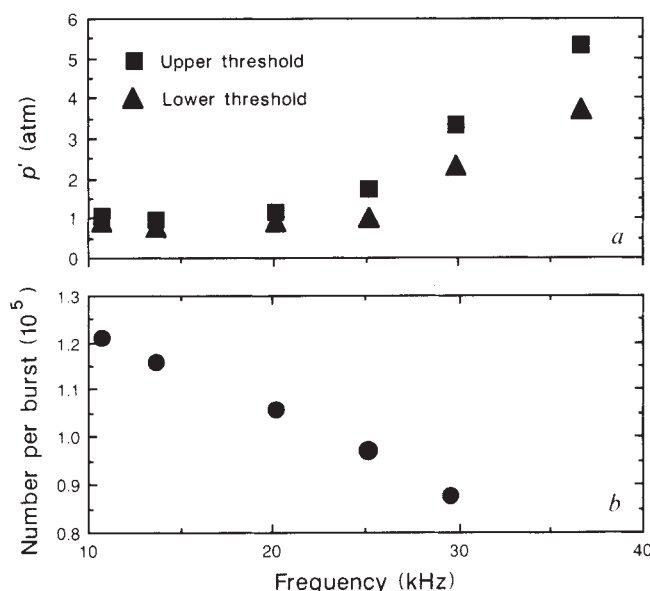


FIG. 1 a, Phase plane for continuous single-bubble sonoluminescence. The 20-kHz data point agrees with the measurement of Gaitan. b, Photons per burst as a function of acoustic frequency; p' has been chosen to maximize the light output.

of the SL flashes with a microchannel-plate PMT (rise time rated as 240 ps). The response is shown in Fig. 2c and d for the SL and pulsed laser sources respectively. Again these responses are virtually identical. The difference between the observed rise time of 290 ps and the rated value is due to detection bandwidth and the variation in pulse heights recorded at the output of the PMT. (Details will be discussed elsewhere: B.P.B., R. Hiller, K. Arisaka, H. Fetterman & S.P., manuscript in preparation.) As Fig. 2d (and b) yields the response to a δ -function source, it can be used to deconvolve the response to SL: Fig. 2c (and a). We thus conclude that the (conservative) upper limit on Δt_{SL} is 100 ps.

The number of photons in a single burst can be determined from the integrated charge output of the PMT, given by the area of the pulse divided by the resistance to ground of 50 Ω through which it flows. Assuming that each electron is created by one photon, the total photon emission is then obtained by correcting for the gain (2×10^7 at 1 kV), the efficiency (electrons per incident photon; $\sim 17\%$) and the solid angle subtended by the active element in the tube ($1/1,290$). The distribution of pulse heights is shown in Fig. 3a. To determine the characteristic shapes in Fig. 2, the trigger was set at a value V_0 above the peak height. Figure 1b shows the intensity of SL as a function of acoustic frequency, f_a . Figure 3b shows the increase in intensity as a function of the amplitude p' of the sound field at a frequency $f_a = 10.736$ kHz.

The clock-like property of SL was investigated by triggering on one SL pulse and using a time delay ($1/f_a$) to acquire the next pulse. Because the scope produces an average of pulses, this procedure increased the rise time, but only by 50 ps. Therefore, we conservatively estimate that the spread in time between successive pulses ($\Delta \tau_{\text{SL}}$ is the standard deviation of $\tau_n - \tau_{n-1}$ where τ_n is the time of emission of the n th pulse of light) is less than 200 ps. The maximum drift in $\tau_n - n/f_a$, recorded over long times, is ± 50 ns provided that the drive pressure and frequency are chosen to lie well within the limits for producing SL (Fig. 1a). Near the upper threshold for SL we have measured drifts of more than 1 μ s. Gaitan¹⁰ has also reported observing regions of parameter space where the phase 'jitter' is less than 1° of an acoustic cycle.

The quality factor Q for the acoustic resonances was determined from the 'ring-down' time of the flask as measured by the microphone. At $f_a = 25.257$ kHz, Q was 1,200, which compares reasonably with the value that one would calculate if the losses were due to acoustic radiation damping at the surface of the flask; $Q_r = \pi \rho u / (2 \rho u)_g \approx 6 \times 10^3$. (Subscript g denotes room-temperature air surrounding the flask and unsubscripted quantities

refer to the liquid.) This should in turn be compared with the quality factor that would apply if the acoustic damping were due only to shear (η) and bulk (ζ) viscosities of the fluid, $Q_\zeta = u^2 \rho / [2 \pi f_a (4 \eta / 3 + \zeta)]$. For water, Q_ζ is $\sim 1 \times 10^7$ at $f_a = 10^4$ Hz. Use of Q_r leads to the acousto-optic conversion efficiency of $\sim 10^{-5}$, whereas use of Q_ζ would imply a conversion efficiency approaching 1%. It would thus be interesting to study SL at low f_a and when the resonator is surrounded by a vacuum so that Q would be influenced by the emission of light and the dynamics of the bubble's motion. The transport properties of the bubble (cavity) will depend upon mass diffusion, thermal conduction to the surrounding liquid, acoustic radiation losses at its boundary and viscous losses. The conversion efficiency of 10^{-5} is similar to the fraction of an external macroscopic stress that is responsible for cumulative damage (fatigue) at the microscopic level in structures^{12,13}. It would therefore be especially interesting to determine whether an increased Q will result in an increased acousto-optic conversion efficiency.

The pulse width of 100 ps or less is a striking result. It should be compared with previous estimates of 6 μ s (ref. 14) and with the assertion that the cavitation hot spots have a lifetime of a few microseconds¹⁵. (Reports¹⁶ of flash widths in the range of 10 ns were brought to our attention by a referee and will be discussed elsewhere; B.P.B. *et al.*, manuscript in preparation.) The flash widths that we find are so short that one wonders whether some phenomenon stimulates the atoms to fire in unison. Known cooperative phenomena include laser action, super-radiance¹⁷ and super-fluorescence¹⁸. Any cooperative phenomenon underlying our observations must be of a spherical nature, however, because a randomly oriented dipole emission would lead to a broad spread in the distribution of pulse heights. According to Fig. 3a, no such broadening is seen. Nevertheless, it is reasonable to expect that some type of correlation characterizes the outgoing photons, because the spacing between light-emitting sources is much less than the wavelength of the emitted light.

To estimate this spacing we return to the standard model for SL and consider the radial oscillations of a bubble of radius R that is bounded by the maximum and minimum values R_{max} and R_{min} . The bubble's contents at the ambient radius R_0 have a temperature of 300 K and a pressure of 1 atm. As the bubble collapses from R_0 to R_{min} it heats and compresses the enclosed gas according to the van der Waals adiabats

$$\begin{aligned} p(R^3 - a^3)^\Gamma &= \text{constant} \\ T(R^3 - a^3)^{\Gamma-1} &= \text{constant} \end{aligned} \quad (2)$$

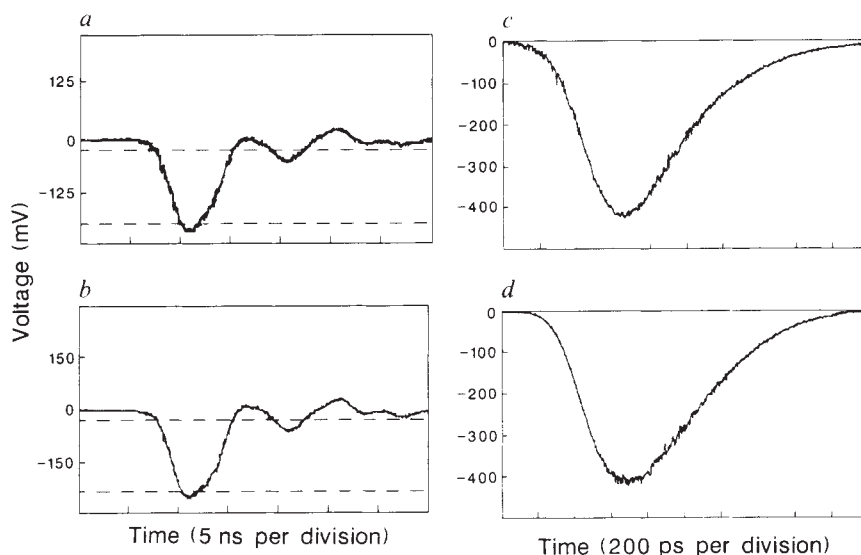


FIG. 2 The average of single-pulse outputs of the photomultiplier tube. a, SL data as recorded by a conventional (R928) PMT; b, same as a, but using a 34-ps laser pulser (Hamamatsu PLP-01) as the light source. a and b were obtained by running the PMT output into a digital sampling scope (HP 54201A). c and d, Data for a microchannel-plate PMT (Hamamatsu R1564U) running into a 20-GHz digital sampling scope (Tektronix 11802). c is for the SL source, and d for the 34-ps laser pulser.

where p is pressure, $\Gamma = C_p/C_v$ ($=1.4$ for air) is to be evaluated in the ideal gas limit and $(4/3)\pi a^3 = nb$ is the van der Waals hard core (n is number of moles, and b is the excluded volume which is $4 \times 10^{-5} \text{ m}^3$ for air).

We estimated the maximum bubble radius to be $\sim 75 \mu\text{m}$ (in a sound field of 1.4 atm) by visually comparing it with a 1-mm -diameter pipette introduced into the fluid. As an example, we take $R_0 \approx 20 \mu\text{m}$, which implies that the bubble contains 8×10^{11} air molecules and that $a \approx 2.3 \mu\text{m}$. During each burst, about one in every 10^7 bubble molecules radiates. As SL requires considerable heating of the contents of the bubble, we expect that $R_{\min} \approx a$, which implies that $R_{\min}$ is about five times the wavelength of light, and the average spacing between emission sites is about one-eighth the wavelength of light. This estimate assumes that the emitters are evenly spaced throughout the volume of the bubble. Because $\Delta t_{\text{SL}} u \leq 0.1 a \ll a$, the entire volume of the bubble may not be able to reach local thermodynamic equilibrium during the rapid adiabatic compression. In this case the hot spot would have thickness $u \Delta t_{\text{SL}}$, and the spacing between emitters would be less than one-fiftieth of the wavelength of light.

It is also possible that the shortness of the light pulses arises from an extremely rapid cooling of the bubble after its oscillation passes through $R_{\min}$, which we will call picosecond adiabatic cooling. A simulation of the Navier-Stokes equations for the above parameters yields $R_{\min} = a + 0.1 \mu\text{m}$ and a bounce time of 400 ps for temperature to decrease by 50% from its value at $R_{\min}$. Details of this simulation will appear elsewhere (R. Löfstedt, B.P.B. and S.P., manuscript in preparation). The assumption that the bubble contains a conserved number of air molecules and is not a vaporous cavity can be tested by spectral analysis¹⁹.

The phenomenon of sonoluminescence represents a failure of the Navier-Stokes equations in a situation where one would normally expect them to apply. The Mach number of the imposed sound field here is much less than one and the wavelength of the sound is very large compared with the microscopic mean free path, yet light is produced, and is emitted, furthermore, in a clock-like manner.

This phenomenon arises from the highly nonlinear oscillation of a bubble which at large R is an ideal gas and at small R is a liquid or solid (or possibly a plasma). The acceleration at $R_{\min}$ is 10^8 times as large as the acceleration at $R_{\max}$. These oscillations involve the transfer and focusing of energy from macroscopic scales down to the molecular, atomic or even electronic degrees of freedom. The huge, spontaneous (non-equilibrium)

amplification factors discussed above are noteworthy in that they are controllable and reproducible. In this respect, stable synchronous SL differs from other phenomena (such as dust explosions, ball lightning and highly speculative conditions for nuclear fusion) that also require large spontaneous energy concentrations. If we could understand the mechanism behind synchronous SL, we might see a way to achieve large but controllable energy concentrations more generally. $\square$

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A synthetic gallophosphate molecular sieve with a 20-tetrahedral-atom pore opening

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THE most widely used catalyst in petroleum cracking and reforming processes is the synthetic zeolite Y. Its unique properties arise from the fact that the enormous inner surface area created by the three-dimensional channel system is accessible to sorbed molecules through 12-ring pore openings. These windows, with free diameters of $7\text{--}8 \text{ \AA}$, allow both aliphatic and small aromatic molecules to enter the zeolite. Attempts to synthesize zeolitic structures with even wider pores, to accommodate larger molecules, have produced two aluminophosphates with one-dimensional channels: VPI-5 (ref. 1), which has 18-ring channels with free diameters of $12\text{--}13 \text{ \AA}$, and $\text{AlPO}_4\text{-8}$ (refs 2, 3), with oval 14-ring channels. We now report the structure of a new cubic gallophosphate with a pore opening comprising 20 tetrahedrally coordinated atoms in the shape of a four-leafed clover (Fig. 1) and a three-dimensional channel system. The unusual shape of the window is due to the presence of terminal hydroxyl groups in the framework, and provides new possibilities for shape-selective sorption. The supercage formed at the intersection of the channels has a body diagonal of $29\text{--}30 \text{ \AA}$, and could thus accommodate larger intermediates in zeolite catalytic processes.

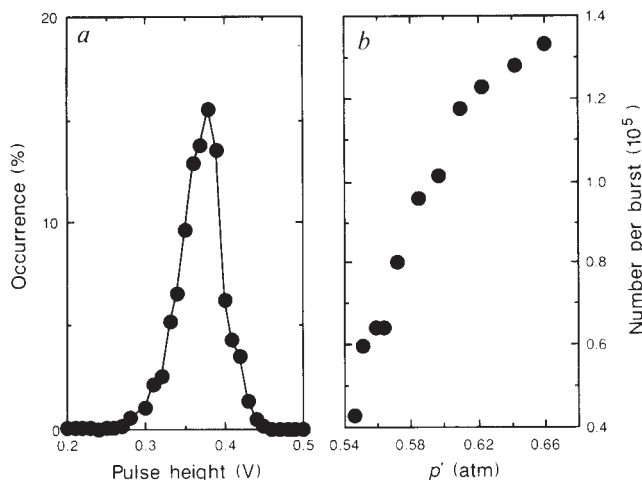


FIG. 3 *a*, Pulse height distribution. A pulse height of 0.1 V represents 1.1×10^4 photons emitted. $f_a = 20.193 \text{ kHz}$. *b*, Photons per burst as a function of acoustic pressure amplitude. $f_a = 10.736 \text{ kHz}$.

The search for new zeolite-like structures was first extended beyond the traditional aluminosilicates to the aluminophosphate-based molecular sieves⁴, followed by explorations of more exotic structures such as beryllio-⁵ and zinco-phosphate and arsenate molecular sieves⁶. Some of these syntheses have produced new zeolite-type structures, including the large-pore aluminophosphates VPI-5 and AlPO₄-8. Only a few gallophosphates with tetrahedral framework structures are known^{7,8}. Expansion of the fluoride synthesis method⁹ to the gallophosphate system has recently produced two new gallophosphate molecular sieves^{10,11}. One has the LTA structure type (A. Simmen, J. Patarin, C.B., A.M. & H.K., manuscript in preparation); the other is reported here.

Preliminary evaluation of this second phase, [Ga₇₆₈P₇₆₈O_{2,976}(OH)₁₉₂] · 192 RF, where RF is quinuclidinium fluoride, indicated that it was both thermally stable and able to sorb large organic molecules. The powder diffraction pattern measured as a function of temperature showed distinct intensity changes accompanying the loss of the organic at ~350 °C, but no significant shift in the lattice parameter or loss of crystallinity until a transformation to GaPO₄-tridymite occurred at ~700 °C. The material calcined at 450 °C sorbed 13 wt% para-xylene and 5.3 wt% tri-isopropylbenzene at 25 °C. To understand these promising properties in more detail, we undertook a structural analysis. High-resolution synchrotron powder diffraction data, collected at wavelength $\lambda = 1.7047$ Å on station 8.3 at the synchrotron radiation source in Daresbury¹², could be indexed unambiguously. Initially, a primitive cubic unit cell with $a' \sim 25.8$ Å was found, but closer examination showed a series of weak superlattice reflections which required a doubling of the lattice parameter. All reflections could then be indexed on a face-centred-cubic unit cell. Several attempts were made to solve the structure from the powder data using the primitive pseudo cell. Both a simple equipartition and a non-standard decomposition of overlapping reflections (M.E., thesis, in preparation) were investigated, but the difficulties arising from the extreme ambiguity of the data (552 of the 617 reflections used were involved in exact 2θ overlaps) could not be surmounted with either method.

Fortunately, the continuing synthesis effort produced single crystals large enough for conventional crystal-structure analysis. These were obtained using a starting composition of 1 Ga₂O₃ : 1

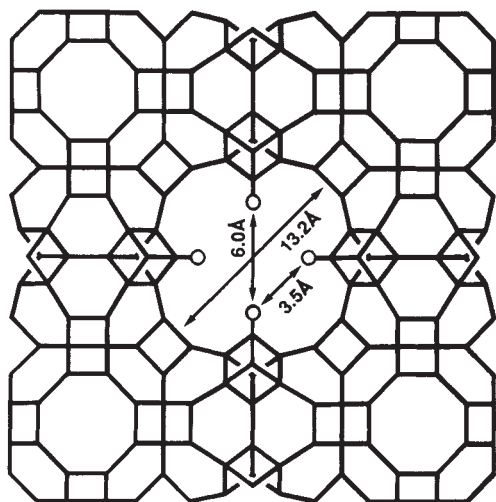


FIG. 1 A projection of the pseudo cell along the [100] direction showing the cloverleaf-shaped 20-tetrahedral-atom pore opening. The nodes represent tetrahedral atoms (either Ga or P), the circles, terminal hydroxyls. The free dimensions of the pore opening, calculated using an oxygen radius of 1.35 Å, are indicated.

TABLE 1 Atomic coordinates and isotropic thermal parameters U

	x	y	z	U (Å ²)
P(1)	0.0888	0.3656	0.2818	0.0172
Ga(1)	0.1341	0.3275	0.2780	0.0139
P(2)	0	0.3226	0.2769	0.0094
Ga(2)	0.0370	0.3507	0.3134	0.0175
P(3)	0	0.3876	0.3414	0.0146
Ga(3)	0.0958	0.3654	0.2236	0.0140
P(4)	0.1336	0.3210	0.2180	0.0078
Ga(4)	0	0.3242	0.2140	0.0150
P(5)	0.0446	0.3097	0.3556	0.0121
Ga(5)	0	0.3415	0.3801	0.0315
O(1)	0.0800	0.3669	0.2542	0.0351
O(2)	0.1059	0.3885	0.2891	0.0179
O(3)	0.1036	0.3404	0.2866	0.0310
O(4)	0.0657	0.3679	0.2999	0.0201
O(5)	0.1360	0.3124	0.2458	0.0506
O(6)	0.1339	0.2970	0.2951	0.0182
O(7)	0.1631	0.3439	0.2914	0.0579
O(8)	0.0237	0.3376	0.2830	0.0434
O(9)	0	0.3158	0.2475	0.0152
O(10)	0	0.2979	0.2916	0.0259
O(11)	0.0521	0.3286	0.3360	0.0537
O(12)	0.0242	0.3795	0.3277	0.0500
O(13)	0	0.3738	0.3670	0.0395
O(14)	0.1091	0.3344	0.2142	0.0406
O(15)	0.0675	0.3692	0.2010	0.0286
O(16)	-0.0289	0.3436	0.2115	0.0354
O(17)	0.0283	0.3231	0.3764	0.0395
O(18)	0	0.4161	0.3461	0.0148
O(19)	0	0.3461	0.4150	0.0759
F(1)*	0.1335	0.3665	1/4	0.0219
F(2)	0	0.3325	0.3297	0.0258

Space group $Fm\bar{3}c$, $a = 52.712$ Å, origin at centre of supercell. U is the thermal parameter.

* z is given as $\frac{1}{4}$ rather than 0.25 because this coordinate is fixed by symmetry and cannot be refined (the same is true for the zeroes).

P₂O₅ : 6 quinuclidine : 0.75 HF : 64 H₂O. This mixture was heated at 150 °C for 24 hours in teflon-lined stainless steel autoclaves. We used gallium sulphate as the source of Ga₂O₃ (A.M., H.K. & J. L. Guth, manuscript in preparation). Data were collected on a 90- μ m crystal using molybdenum K α radiation ($\lambda = 0.71073$ Å, graphite monochromator). The space group $Fm\bar{3}c$ could be assigned uniquely after a careful inspection of all reciprocal lattice points for which $2\theta < 45^\circ$. A total of 14,340 reflections were measured and then merged to yield 2,776 independent reflections. Of these, the 1,174 with intensity I more than 3 times the standard deviation at I were used in refinement. The framework structure in the $Pm\bar{3}m$ pseudo cell approximation was solved by direct methods with the program SHELXTL-Plus¹³.

Once the framework topology had been established, the average tetrahedral atom positions in the pseudo cell were transformed to the larger cell and to the space group $Fm\bar{3}c$ to allow for the anticipated strict alternation of Ga and P in the framework. Distance-least-squares calculations with the program DLS-76¹⁴ yielded the optimized desymmetrized atomic coordinates and thus gave starting phases for the superlattice reflections. The positions of the terminal oxygens (O(18) and O(19) in Table 1), where the framework is not fully connected, and of the F⁻ ions used in the synthesis, were located in the first-difference Fourier map. Isotropic refinement of this structure resulted in the residuals $R = 0.105$ and $R_w = 0.160$ (ref. 15). Location of the organic material in the channels and cavities proved to be nontrivial and is still in progress. When the 41 lowest-angle reflections, which are most sensitive to these non-framework atoms, were removed from the refinement, the error indices R and R_w dropped to 0.087 and 0.076 respectively. Atomic coordinates from this refinement are given in Table 1.

The framework can be described as a cubic arrangement of α -cages connected along each of the edges of the cube by two *rpa*-cages (Fig. 2). Both of these cages are found in other zeolite structure types: the α -cage in KFI, LTA, LTN, PAU and RHO¹⁶, and the *rpa*-cage in $\text{AlPO}_4\text{-22}$ (AWW)¹⁷. All the four-membered rings of these cages are involved in double four-rings (D4R). In fact, the entire structure can be built from D4Rs. Half of these D4Rs are not fully connected, and the coordination spheres of a Ga and a neighbouring P in these cubes are completed with terminal oxygens (presumably hydroxyl groups). Thus, this material has an interrupted framework, as do the minerals chiavennite, parthéite, roggianite and wenkite.¹⁶ The framework

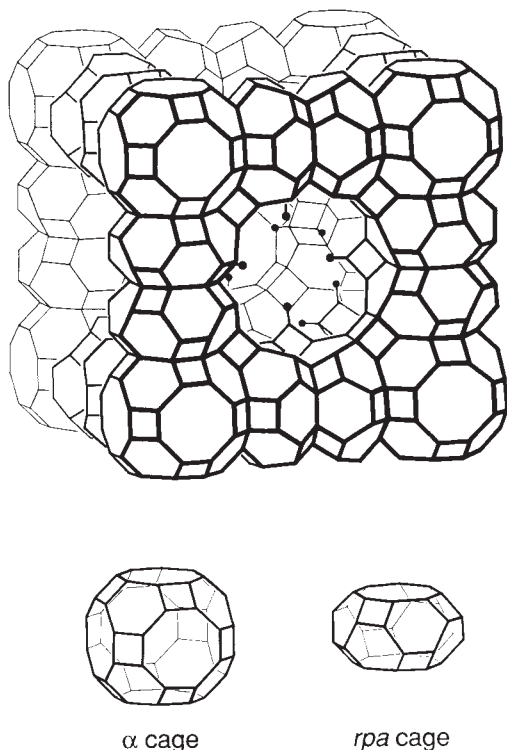


FIG. 2 The framework topology ($Pm\bar{3}m$) of the new gallophosphate molecular sieve showing the cubic arrangement of α - and *rpa*-cages. The nodes represent tetrahedral atoms (either Ga or P), the filled circles, terminal hydroxyls. For clarity, the α - and *rpa*-cages are also shown separately.

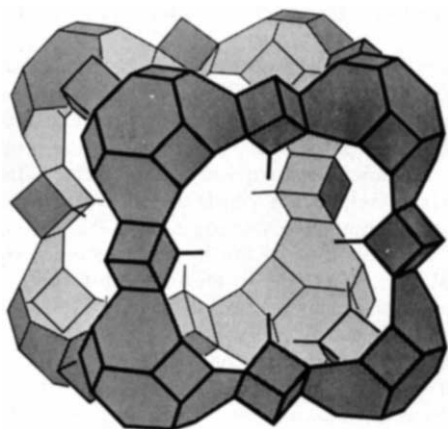


FIG. 3 A skeletal drawing of the large supercage of the framework structure showing the cloverleaf-shaped windows and the pockets at the corners of the cage.

has two nonintersecting three-dimensional channel systems. One of these passes through the α - and *rpa*-cages and has eight-ring pore openings, and the other passes through the face of the cube described above and has a cloverleaf-shaped pore opening described by 20 tetrahedral atoms (Ga and P) and 24 oxygens (Fig. 1). The shape of the channel opening is dictated by the fact that the D4Rs with terminal oxygens protrude into the window. At the intersection of these channels is a large cubic supercage (Fig. 3) with pockets at the corners. The body diagonal from pocket to pocket is 29–30 Å.

All the D4Rs in the structure have a fluoride ion incorporated in the centre. The D4Rs are distorted in such a way that the F^- ion can approach all four Ga atoms at distances ranging from 2.30 to 2.66 Å. The Ga atoms, in turn, become five-coordinate with distorted trigonal bipyramidal coordination. The P–F distances are all greater than 2.78 Å, and the P atoms retain their tetrahedral geometry. A similarly distorted D4R is found in the gallophosphate-LTA, the structure of which can also be built entirely from D4Rs. Interestingly enough, the pure silica form of the AST structure¹⁸, which was also synthesized with the fluoride method, and which also has a preponderance of D4Rs, also has a F^- ion in each of the D4Rs.

This new gallophosphate molecular sieve has an extremely open structure. Its framework density is only 11.1 tetrahedral-atoms per 1,000 Å³, compared with 12.7 for FAU. Brunner and Meier¹⁹ suggested that one way of making very-low-density tetrahedral framework structures was to incorporate three-rings into the framework. Apparently, forming an interrupted framework with a robust support structure is another. The high thermal stability of this material can probably be attributed to the sturdiness of the α - and *rpa*-cages and to the particular stability of the fluoride-containing D4Rs. Investigation of the stability (and acidity) of the hydroxyl groups themselves is in progress.

Several features of the structure of this new molecular sieve could be exploited. The unusual shape of the pore opening restricts the size and shape of molecules that can be sorbed or desorbed such that large molecules must be flat (at least at their edges) to pass through the window. On the other hand, the large supercage provides space for bulky intermediates to form during a catalytic reaction, or for large molecules, such as iron-phthalocyanine, to be synthesized *in situ*. Such molecules can act as catalytic sites within the confines of the zeolite framework, and be used to mimic enzyme activity²⁰. Because the channel system is three-dimensional, blockages due to coking are less of a problem than in one-dimensional channel systems, and molecules synthesized *in situ* are more easily accessible. Given the right conditions, the terminal hydroxyl groups might be active sites for catalytic reactions.

In view of the outline of the large channel opening and in the hope that it bodes well for the future, we propose the name cloverite for this new gallophosphate molecular sieve. □

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Distribution of dissolved iron in sediment pore waters at submillimetre resolution

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MUCH effort has been directed at measuring concentration gradients at the sediment/water interface of aquatic systems, where the biogeochemical cycling of natural and pollutant species is particularly active¹. Precise measurements of oxygen gradients using microelectrodes² and estimates from independently determined fluxes³ suggest that concentration gradients in this region often extend only to depths of ~1 mm, much less than the resolution (~1 centimetre) of conventional techniques^{4–7}. We have developed a new method for measuring pore-water composition in which diffusive equilibrium is established rapidly (within minutes) in a thin film of gel inserted in the sediment. On removal, the dissolved components are fixed, allowing chemical measurements to be made at high spatial resolution (<1 mm) on a stable solid phase. Using MeV-proton-induced X-ray emission (PIXE) to analyse the dried gel, we have measured iron concentrations in lacustrine pore waters at submillimetre resolution, revealing steep concentration gradients and sub-surface maxima consistent with a hypothesis of localized, reductive dissolution of fresh material.

The sediments of most lakes contain 3–5% iron (dry weight), and in productive, neutral lakes where decomposition processes ensure that pore waters are devoid of oxygen, Fe(II) is typically present in solution at concentrations of 1–20 mg dm⁻³ (ref. 8). If the overlying water contains oxygen, Fe(II) is removed by oxidation to insoluble Fe(III), and a concentration gradient, along which diffusion occurs, is established near to the sediment/water interface³. The location of the site of oxidation depends on the precise position of the redox boundary, but formation of iron oxides a few millimetres below the sediment surface has been observed *in situ*⁹. This vertical specificity of the deposition product indicates that the gradient supplying Fe(II) by diffusion probably only extends over millimetre intervals of depth, as is estimated from indirect calculations of the diffusion layer thickness³.

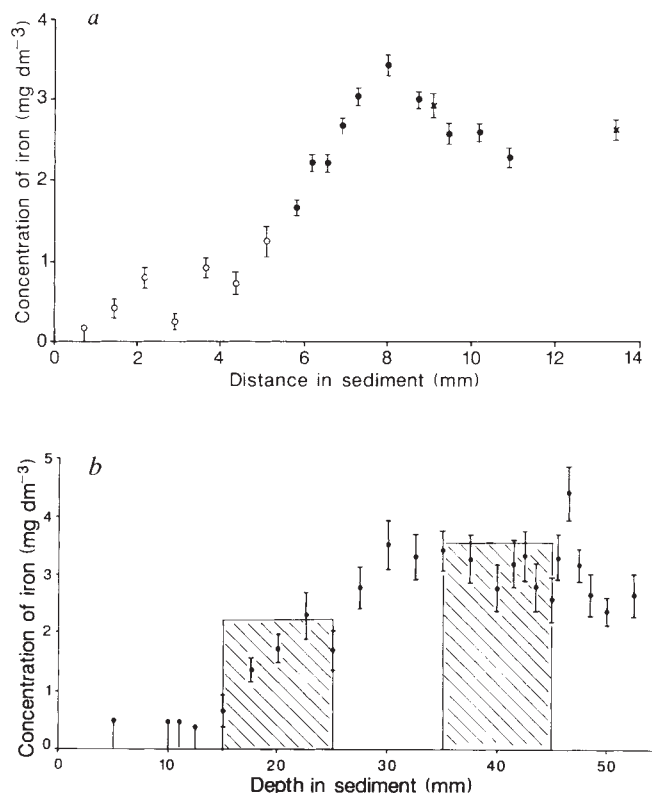
Dialysis cells in which a membrane separates distilled water from the sediment have been shown to provide good estimates of pore-water concentrations^{5–7}. After the cells have been immersed in the sediment for a few weeks, diffusive equilibrium is established, and the enclosed solution assumes the same composition as the pore waters. Dialysis techniques only provide a few millilitres of solution for analysis and construction difficulties make them difficult to scale down. The technique of diffusive equilibration in a thin-film (DET) relies on a similar equilibration principle, but rather than confining the solution to compartments, it uses a thin film of gel to provide the medium for

solution equilibration. A thin film is conveniently prepared, stable, and has the further advantage of requiring a relatively short time for solution equilibration. Fick's laws indicate that for a typical dialysis cell (1 cm deep), complete equilibrium is established in 3 days if we assume a diffusion coefficient of 10⁻⁵ cm² s⁻¹. Using a 1-mm-thick gel with the same diffusion coefficient, complete equilibration would take only 42 minutes.

A polyacrylamide gel composed of 15% by volume acrylamide (Boehringer) and 0.3% by volume AcrylAide cross linker (Flow Gen Instruments Ltd, UK) was used. It was cast between two glass plates separated by plastic spacers to give final dimensions of 3.1 × 15 × (0.08 or 0.10) cm, depending on the thickness of the spacers. The gel system used is similar to those described for the electrophoretic separation of proteins¹⁰ and does not impede the diffusion of simple ions such as Fe(H₂O)₆²⁺. Once the gel was set, the top plate was removed and replaced with a 2-mm-thick acrylic plate which had a central front window (1 × 10 cm) and was held in place by plastic clips. To remove oxygen, the gel was equilibrated for 24 h in water bubbled with carbon dioxide. The gel system was then immediately inserted into the sediment until the front window was half exposed to the sediment and half to the overlying water. After equilibration for 24 h, it was removed from the sediment and rinsed briefly (for 3 s) with distilled water before immersion for 2 h in a solution of 1 mmol l⁻¹ sodium hydroxide solution. At this high pH, the oxidation of Fe(II) is virtually instantaneous¹¹. Therefore, as oxygen and hydroxyl ions diffuse into the gel they will convert Fe(II) into insoluble and immobile Fe(III). Although some iron will inevitably be lost, most will be retained in an immobile form. After this treatment the gel was washed, peeled from its backing plate and then pressed between either Teflon or acrylic sheets before drying at 60 °C. The gel shrank on drying, and the new thickness of 50–100 µm was measured by scanning electron microscopy to provide a concentration factor for each film. Iron concentrations in the dried gel were determined using PIXE¹² at the Oxford Scanning Proton Microprobe^{13,14}. Because iron, manganese and other diagenetic products would be precipitated *in situ* on the front surface of the gel⁹, measurements were made from the back of the gel, which had not been exposed to the sediment. Calculations of Fe K α X-ray production and adsorption show that when 2-MeV protons are used, the contribution from any iron precipitated on the surface of the gel is negligible provided that the gel thickness is >50 µm. Concentrations in the pore waters were calculated from the measured volumetric concentration factor and the density of the gel, assuming that 90% of the wet gel consisted of water capable of equilibrating with the pore waters.

Jenkin cores¹⁵ of the sediment/water interface were taken from the deepest parts of two productive lakes in winter when the overlying waters were well oxygenated. Gels were inserted within 2 h of collecting the cores. A standard was provided by inserting a gel into a deoxygenated solution of 2.4 mg dm⁻³ Fe(II) in an anaerobic chamber. The concentration of the standard solution estimated from analysis of the dried gel was 2.3 ± 0.7 mg dm⁻³. Measurements of iron in pore waters extracted with a syringe⁴ from a duplicate core also agreed well (Fig. 1b). Blanks were prepared by leaving the gels in deoxygenated water for 24 h, all other treatments being identical. Scans over 3-mm squares showed the concentration of iron to be <0.2 mg dm⁻³ in the wet gel. Iron concentrations measured in both pore waters had steep gradients (Fig. 1) which could not have been resolved using other established procedures, and both profiles had sub-surface maxima. The latter feature is consistent with previously proposed mechanisms for redox cycling³. Iron, supplied to the sediment from sinking particles, effectively moves downwards as new material is recruited to the sediment. A point source of dissolved Fe(II) develops at the location in the sediment where there are electron donors capable of reducing the reactive fraction of the Fe(III). Iron(II) diffuses upwards and downwards away from this point source. The downward-

FIG. 1 Vertical iron distributions in the pore waters of *a*, Esthwaite Water¹⁶ on 4 December 1990 and *b*, Mitchell Wyke, Windermere¹⁷ on 15 January 1991. The original cast thicknesses of the gels were 0.79 mm (*a*) and 1.00 mm (*b*). For (*a*) the dried gel had to be cut before microprobe analysis: different symbols indicate measurements made on different pieces of gel. The sediment/water interface (0 cm) was not estimated accurately. For (*b*) the sediment/water interface was estimated visually (± 1 mm), and concentrations measured on a duplicate core using an alternative technique⁴ are shown by hatching. The dried gels were analysed using a beam of 2 MeV protons focused to 1 μ m diameter with a beam current of 200 pA. The beam was scanned over an area of $100 \times 100 \mu\text{m}$ at each position to reduce radiation damage and to average out micrometre-scale inhomogeneities in the sample. The emitted characteristic X-ray spectrum from each area was recorded and processed to give iron concentrations. The total proton dose at each point was $0.02 \mu\text{C}$, giving a minimum detection limit for iron of $<10 \mu\text{g}$ per g in the dried gel. Error bars represent the analytical error of the microprobe and points on the baseline are less than the detection limit.



diffusing iron is regulated by diagenetic reactions, solubility and interactions with surfaces. The upward-diffusing iron is oxidized and removed from solution as new solid phases are formed. Using a linear approximation for the slope of the diffusion gradient, we estimate the upward flux of iron to be 1.5×10^{-12} and $3.1 \times 10^{-13} \text{ mol cm}^{-2} \text{ s}^{-1}$ for Esthwaite Water and Windermere respectively.

In the summer months the water overlying the sediments from the deeper parts of Esthwaite Water is devoid of oxygen, allowing Fe(II) to diffuse from the sediments without oxidation. The time-averaged flux of $\sim 1 \times 10^{-12} \text{ mol cm}^{-2} \text{ s}^{-1}$, estimated from the accumulation of iron in the bottom waters^{18,19}, is similar to the single winter value measured here. This observation indicates that iron is remobilized throughout the year, but it is more difficult to observe in the winter when the release is not obvious, as the overlying waters are oxygenated and well mixed.

The sharp peak (5 mm wide) in the concentration of iron observed at a depth of 46.5 mm in Windermere sediment (Fig. 1*b*) may well be real, because the spatial resolution was sufficient to show its systematic rise and fall. It probably reflects the heterogeneity of the sediment and indicates that there may be microniches of high reducing activity that serve as a local source of Fe(II).

The realistic spatial resolution of the technique is determined partly by diffusional relaxation in the film during equilibration in the sediment and partly by the diffusional relaxation time between removal of the film from the sediment and fixation. As used here, diffusional relaxation expresses the tendency for ions in the gel to diffuse from regions of high to low concentration, so obscuring concentration gradients. Diffusional relaxation during equilibration is determined by the film thickness, which could reasonably be reduced to 100 μm . With the present procedure, the fixation time also depends on the film thickness, as this determines the time it takes O_2 and OH^- to diffuse into the film. Thus the best spatial resolution is roughly equivalent to the film thickness, which in this work was ~ 1 mm. Measurements made with the microprobe at finer intervals will contain an element of smoothing due to diffusional relaxation. Preliminary experiments have shown that fixation may be possible using

immediate freezing in liquid nitrogen followed by freeze drying. For this procedure, a realistic solidification (and hence fixation) time of 5 s would correspond to a spatial resolution free from smoothing of 100 μm , as estimated from the mean diffusional path length.

This diffusive equilibrium technique could therefore measure dissolved iron in pore waters at previously unattainable resolution. As for dialysis techniques, the gels could be inserted *in situ* by divers. The accurately measured concentration gradients may be used to calculate fluxes at the sediment/water interface. When linked to measurements of *in situ* solid-phase formation⁸, the DET technique should provide the data necessary to model quantitatively the processes of iron dissolution and precipitation. Moreover, the technique need not be restricted to iron. With a general fixation procedure, such as freeze drying, any freely diffusing component can be measured, provided that it is sufficiently concentrated. By incorporating chemical concentration steps within the gel, it should be possible to lower the detection limits and hence measure more dilute components. The DET technique is not in principle restricted to lake sediments. It could be applied to soils or any water where convection is limited. Its short equilibration time allows it to be used in dynamic situations. For example, it could be used to measure the variation in sediment-water fluxes during river flooding. □

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***In situ* chemical mapping of dissolved iron and manganese in hydrothermal plumes**

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HYDROTHERMAL vents along mid-ocean ridges are an important source of elements such as lithium, silicon, manganese and iron to the world's oceans¹. The venting produces both episodic and steady-state hydrothermal plumes with unique thermochemical signatures in the mid-water column. The particulate phases in these plumes (predominantly iron oxides and hydroxides) also scavenge phosphorus, vanadium, arsenic, lead, polonium and several rare-earth elements from sea water^{2–5}. Thus, on a global scale, hydrothermal plumes are both a source for some elements and a sink for others. Ultimately, the particulate metals precipitated from plumes form extensive regions of metalliferous sediments over the crests and flanks of mid-ocean ridges^{6,7}. Although the metalliferous sediment coverage is vast and well documented, only a tiny fraction of the vents responsible for these sediments have been located (Fig. 1a). To date, both the number and location of hydrothermal vents and the detailed distribution of chemical constituents within the resultant plumes are poorly understood because of under-sampling of the mid-ocean ridges and the overlying waters. Here we present the results of high-resolution mapping of the chemical and thermal characteristics of hydrothermal plumes in near real time using a novel submersible chemical analyser (Scanner)^{8,9} and a conductivity/temperature/depth/transmissometer instrument package (CTDT)¹⁰. We show that the kinetics of iron oxidation in the plume can be used to constrain estimates of the plume's age, and that variation in the ratio of manganese content to excess heat can be explained by the mixing of several different vent fluids.

Measurements were made during Leg 1 of the VENTS 1989 cruise (27 July to 25 August, 1989) aboard the RV *Discoverer*. The Scanner was secured to a CTDT/rosette package and towed across the Cleft segment at the southern end of the Juan de Fuca Ridge (Fig. 1b and c). *In situ* measurements of dissolved manganese and total dissolved iron (Fe(II) plus Fe(III)) were performed continuously, and the data were recorded every 5 seconds. The instrument package was raised and lowered repeatedly¹¹ while the ship steamed slowly at a speed of 1–2 knots. Dissolved manganese was analysed by colorimetry using 1-(2-pyridylazo)-2-naphthol (PAN)¹², and total dissolved iron was determined with ferrozine¹³ after reducing Fe(III) to Fe(II) with ascorbic acid. A filter on the sample inlet allowed only dissolved metals (<10 µm) to be analysed. Simultaneous measurements of conductivity, temperature, depth and light attenuation (an indicator of hydrothermal plume particles) were also obtained with the CTDT mounted on the rosette¹⁰. In addition, 10 discrete samples were obtained with the rosette on

each tow. These samples were analysed for dissolved (<0.2 µm) and particulate manganese and iron¹⁴. Similar results for determinations of dissolved metal performed *in situ* and in the laboratory (see below) indicate that the colorimetric analyses used *in situ* are insensitive to metal in the particulate phase.

The track of the Scanner/CTDT package across the ridge crest during the XT-10 tow, and the corresponding contours of the hydrothermal temperature anomaly, light attenuation, manganese and iron are shown in Fig. 2a–d. This tow produced the first high-resolution survey of manganese and iron in a vent plume. More than 3,700 sets of analyses for both metals were performed *in situ* during this tow. The contoured section of manganese shows excellent agreement with the distributions of temperature anomaly and light attenuation. The highest metal concentrations (259 nM for manganese, 75 nM for iron) were found at a depth of ~2,000 m (260 m above the bottom) and were centred over the axial valley of the ridge.

The correspondence between the dissolved iron contours and other physical parameters was poor (Fig. 2c). A plot of dissolved iron against temperature anomaly for XT-10 (Fig. 3a) has a slope of only 900 nM °C⁻¹ (correlation coefficient $r^2 = 0.45$). The corresponding ratio of iron concentration to excess heat anomaly (Q) is 0.90 nmol cal⁻¹, whereas in hydrothermal-source fluids from vent fields ~25 km further south on the Cleft segment, this ratio (corrected for changes in heat capacity¹⁵) ranges from 22.9 to 78.0 nmol cal⁻¹ (ref. 16). About 97% of the original dissolved iron has been removed from this plume if the source fluids resemble those of the south Cleft segment. This accounts for the weak correlation of iron with other parameters.

A regression plot of manganese against temperature anomaly shows a linear relationship with a slope of 3.78 nmol cal⁻¹ ($r^2 = 0.96$, Fig. 3b), in good agreement with the Mn: Q ratio determined previously for steady-state plumes in this area (1.9–4.1 nmol cal⁻¹)¹⁷. The linear relationship suggests that manganese is not being rapidly removed. The Mn: Q ratio measured in this plume is, however, lower than that found in any of the black smoker vents that have been sampled at south Cleft (4.7–15.0 nmol cal⁻¹)^{16,18}. Either the composition of the black smoker source vents for the plume sampled during the XT-10 tow differs from previously measured values (from the south Cleft segment), or there must be an additional source of water with low Mn: Q that is entrained in the plume. The effects of these processes can be resolved by examining data from other tows in this area.

The results from a vertical cast (X-4, Fig. 3c and d) at a site 12 km to the north show considerably different trends, particularly for iron, from those found during XT-10 (Fig. 3a and b). The trends in the metal/temperature-anomaly plots at both stations are confirmed by the excellent agreement between the *in situ* and laboratory analyses of dissolved manganese and iron (Fig. 3, large symbols). The metal: Q relationships at X-4 are clearly not linear. In addition, the highest Fe: Q ratio at station X-4 (10.6 nmol cal⁻¹) was 12 times as great as the average value found during the XT-10 tow. The high iron concentrations indicate that the plume at X-4 was considerably younger than that sampled during XT-10. Analyses of the rosette samples show concentrations of particulate iron collected during the XT-10 tow that are as high as 475 nM (98% of the total concentration of dissolved and particulate iron in this discrete sample), whereas during X-4 they reached only 226 nM (29% of the total concentration). Estimates of the ages of the plumes at the time of sampling were derived using the iron-oxidation-rate law¹⁹ corrected for temperature, pH and salt effects, the observed Fe(II) concentrations²⁰ and the assumption that no particulate iron had been lost from the plume. The iron-oxidation-rate constant of $1.29 \times 10^{-3} \text{ min}^{-1}$ was derived from the equations of Millero and co-workers¹⁹ for pH (7.74), temperature (1.7 °C) and oxygen concentration (70.1 µM) in the plume. We further corrected for the time between bottle closure and sample acidification (9.56 and 11.76 hours for XT-10 and X-4 respec-

tively). Average $\text{Fe(II)}/\text{Fe}_{\text{total}}$ of 0.0135 and 0.106 yield plume ages of ~46 and 17 hours for XT-10 and X-4, respectively. Although some iron may have been removed in the lower ascending plume, this removal is thought to take place within the first 25 m, or first few minutes of mixing²¹. These ages, although tentative, demonstrate the usefulness of $\text{Fe(II)}/\text{Fe(III)}$ as an age tracer.

The highest Mn:Q ratios at X-4 (6.7 nmol cal⁻¹) are within the range of values that have been found in black smoker vents at the south Cleft Segment (4.7–15.0 nmol cal⁻¹)^{16,18}. The lowest Mn:Q ratios at X-4, however, are well below this range. Chemical scavenging cannot remove enough manganese to produce these low ratios. There is no evidence for significant manganese removal in the buoyant plume at the Juan de Fuca Ridge, at the East Pacific Rise^{21–23} or in the near-field plume at XT-10 (Fig. 3b). Furthermore, bacterial removal of manganese is not rapid enough to account for this deficiency²².

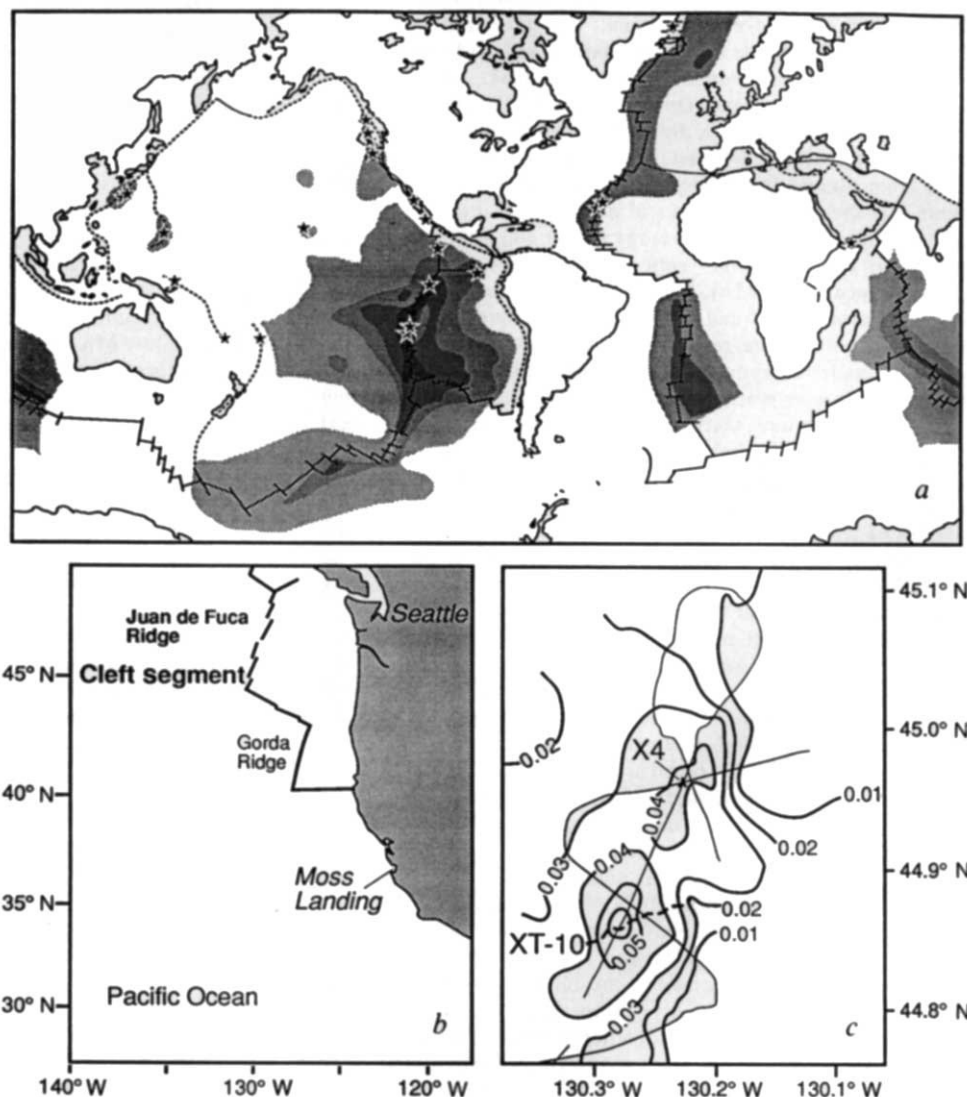
We postulate that the curvature in the relationship between manganese and excess temperature for X-4 is produced by mixing of both the high Mn:Q discharge from black smoker vents and a low-Mn:Q discharge. There are several possible sources for the low-Mn:Q water. Venting of fluids that have undergone phase separation and segregation could provide a low Mn:Q signature to these plumes²⁴. Phase-separated fluids have been sampled from Axial Volcano, which is located between the Cleft and Endeavour segments on the Juan de Fuca Ridge^{24,25}. The condensed vapour-phase fluids have distinctively low chloride concentrations and are depleted in metals relative

to heat (Mn:Q = 0.61 nM cal⁻¹; Fe:Q = 0.03 nM cal⁻¹)^{24,25}. Low-temperature interactions between water and rock, in which sea water is heated but fewer metals are extracted from the basalt, could also produce a low Mn:Q signature²⁶. The mixing of ambient sea water with varying proportions of black smoker fluids and condensed vapour phase or low-temperature fluids could produce the signals observed both at XT-10 and X-4.

Alternatively, episodic large-scale venting, which has produced two megaplume events in this area^{18,26}, could have influenced our observations. These megaplumes are characterized by Mn:Q values (0.2–0.7 nmol cal⁻¹)^{18,27} that are much lower than the steady-state plumes in these areas (1.9–4.1 nmol cal⁻¹)¹⁷. Although XT-10 shows some characteristics of rapid, megaplume venting (homogeneous Me:Q, oblate spheroid shape, higher in the water column), the overall magnitude of this feature was small in comparison with a full-scale megaplume event and the metal:heat ratios were higher.

XT-10 seems to be the result of a rapid discharge from a basically homogeneous source. In contrast, the inhomogeneity in the thermochemical signatures of X-4 indicates not only that there are multiple sources for this plume, but also that the sources must be in close spatial and temporal proximity to be entrained in the same portion of this relatively young and turbulent plume. Hydrothermal fluids with distinct Mn:Q ratios must be present in the vent field that produces this plume. Recent submersible dives by one of us (G.J.M.) have confirmed the presence of both a low- and high-Mn:Q source in this area, conforming to the ratios found in the overlying plume²⁹. It may

FIG. 1 a, Map of the world's oceans showing locations of known hydrothermal vents (stars, showing scarcity of known vent sites), and the distribution of metalliferous sediment coverage where $\text{Al}/(\text{Al} + \text{Fe} + \text{Mn}) < 60$ (indicating vast extent of hydrothermally derived sediments, after Bostrom²). b, Location of the Cleft segment along the Juan de Fuca Ridge. c, Areal map of the hydrothermal temperature anomaly (in °C) at the north Cleft segment and tow track of the CTD package used to map the plumes in this area. The XT-10 tow track is shown by dashed line. The axial valley orientation is N 20° E and directly underlies the temperature anomaly maxima.



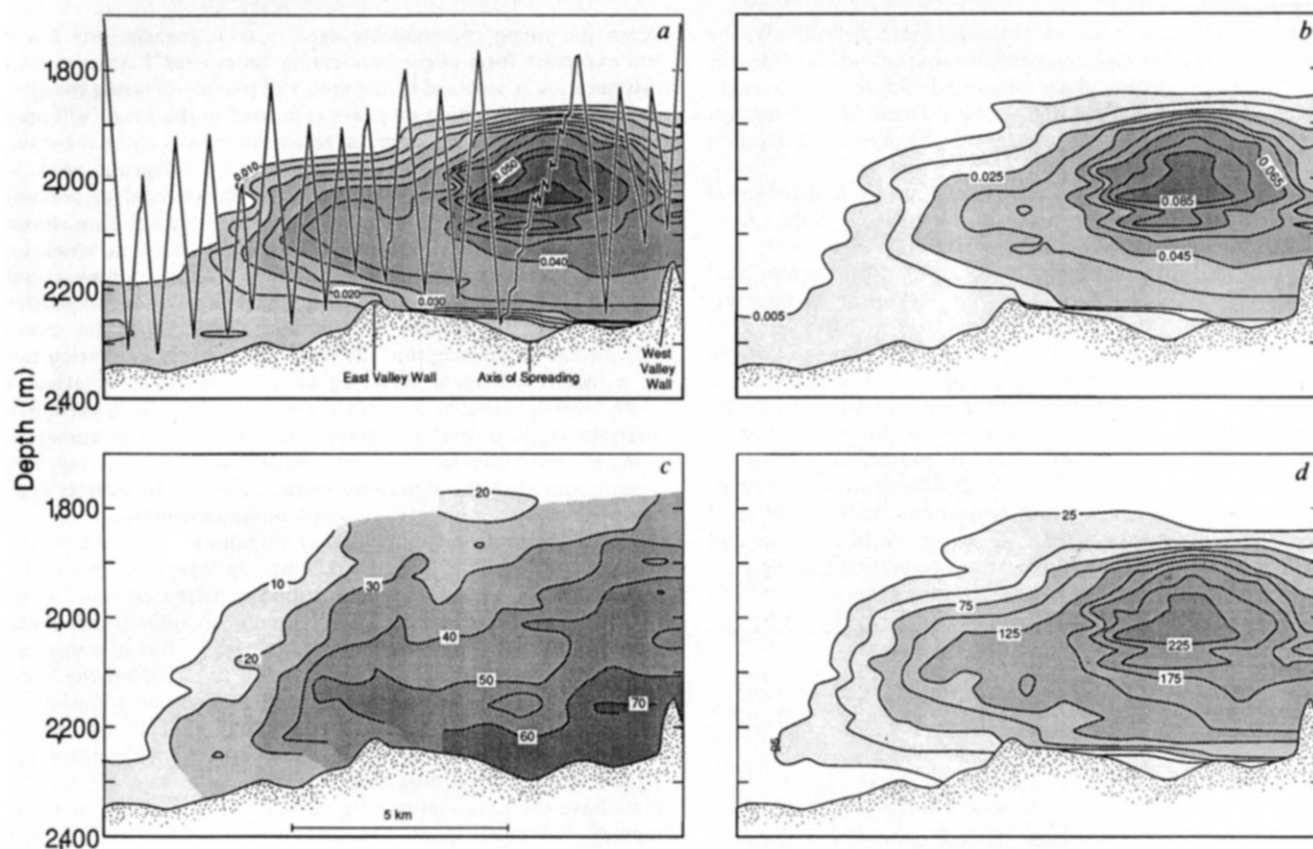
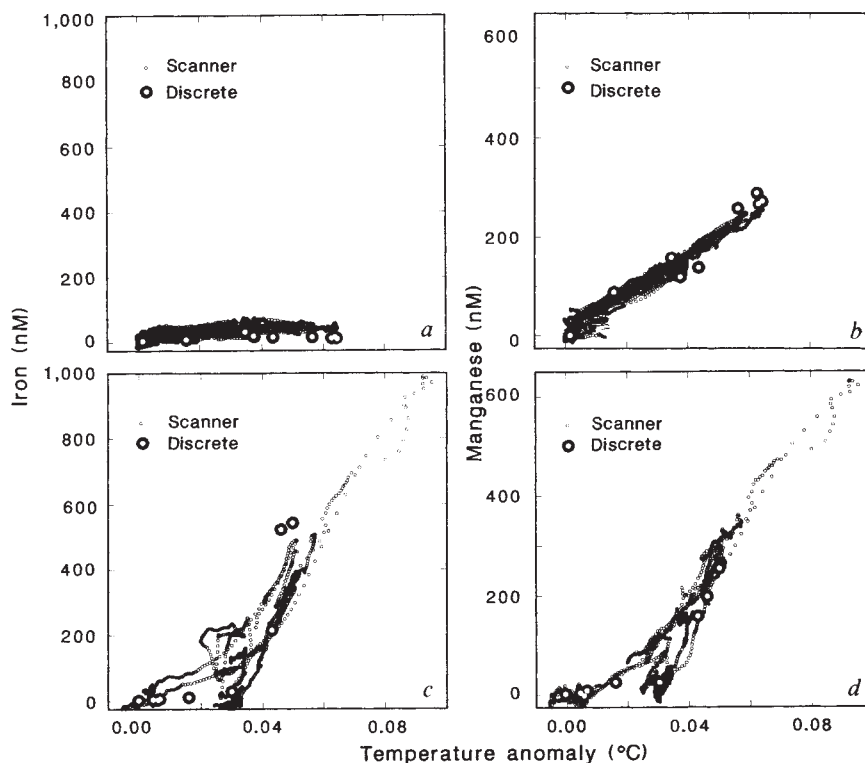


FIG. 2 Measured properties for the tow XT-10, contoured over the tow track. Left-hand side of each panel represents $44^{\circ} 52.8' \text{ N}$, $130^{\circ} 13.0' \text{ W}$ on the eastern flank of the ridge; right-hand side of each panel represents $44^{\circ} 50.6' \text{ N}$, $130^{\circ} 21.0' \text{ W}$ over the western valley wall. *a*, Tow track of the CTD/Rosette/Scanner package, as it was towed in a southwesterly direction across the axial valley (see Fig. 1c), is plotted together with the contoured section of the excess temperature anomaly¹⁰, an indicator of the plume's location and strength. The contour interval is 0.01° C . *b*, Light attenuation anomaly, as measured with a beam transmissometer secured to the CTD package, shows a high correlation between the distribution of hydrothermal

particles (primarily iron oxyhydroxides) and the temperature anomaly. The contour interval is 0.01 m^{-1} . *c*, Iron concentrations contoured over the XT-10 transect at 10-nM intervals show weak correlation with the other conservative tracers (the temperature anomaly and manganese) because of its rapid oxidation and removal. These concentrations are near the Scanner detection limit for iron (25 nM). The noise, therefore, represents both plume heterogeneity and analytical variability at these low concentrations. *d*, The more conservative tracer, manganese, contoured over the XT-10 section at 25-nM intervals shows excellent agreement with the temperature anomaly and light attenuation. The detection limit is 22 nM .

FIG. 3 Plots of dissolved metals against temperature anomaly for the stations XT-10 and X-4. Scanner metal analyses were performed by *in situ* colorimetry¹², whereas discrete dissolved samples were analysed by column extraction followed by graphite furnace atomic absorption spectroscopy (GFAAS)¹⁴ at Pacific Marine Environmental Laboratory (PMEL). Particulate samples were analysed by X-ray fluorimetry using thin-film procedures, also at PMEL. *a*, Iron (nM) against temperature anomaly ($^{\circ} \text{C}$) along XT-10 shows a very low slope relative to the source fluids. The variability in the data reflects concentrations near our detection limit for iron and the nonconservative behaviour of iron in these plumes. The concentrations measured *in situ* agree well with the GFAAS results determined at PMEL. *b*, Manganese (nM) against excess temperature anomaly ($^{\circ} \text{C}$) from both the Scanner and discrete samples from XT-10. This relationship between Mn and temperature anomaly is quite linear ($r^2 = 0.96$) with a slope of $3.78 \text{ nmol cal}^{-1}$, less than that observed in the nearest known source fluids. *c*, Iron against temperature anomaly, plotted for X-4. *d*, Manganese against temperature anomaly, plotted for X-4. The plot for this station shows much more curvature, indicating inhomogeneous mixing of multiple sources, some of which have Mn:Q ratios similar to those observed in the black smokers to the south (see text).



also be possible to use the to evaluate, more definitively, the inputs from low- and high-temperature sources and distinguish the contribution from phase-separated fluids. At present, however, there is no *in situ*, high-resolution method of determining ^3He , and manganese is thought to behave similarly and conservatively in the near field^{21–23}.

In situ determinations of iron and manganese in hydrothermal plumes, coupled with high-resolution CTD data, have increased our understanding of the variability and distribution of metal:Q ratios, typically under-represented by conventional point-source sampling techniques. Thermochemical characterization of hydrothermal plumes in this way may preclude, to some extent, the need for submersible search and verification of the source fluids. The Scanner configuration is flexible and can accommodate one channel for the determination of Fe(II) and the other for Fe_{total}. This potential for the Scanner to be used for *in situ* age determinations in plumes is promising. Furthermore, this technique demonstrates the feasibility of mapping iron and manganese concentrations and metal:heat relationships over mid-ocean ridges of the world's oceans and could therefore provide a more accurate accounting of the global cycling of elements through hydrothermal systems. □

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Signalling of need by offspring to their parents

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THE young of birds and mammals often solicit food from their parents in ways that appear to be costly and to reduce their fitness^{1,2}. Thus birds in the nest beg vigorously for food, incurring energetic costs and possibly attracting predators³; the behaviour of young mammals requiring to suckle (bleating or crying, for example) similarly appears costly^{1,2}. If solicitation is a means by

which the young communicate need to their parents, why has a less expensive form of communication not evolved⁴? An answer to this question is provided by the theory of parent-offspring conflict: natural selection acting on genes expressed in the young will lead to greater demands for parental resources than is optimal for the parent^{1,5}. The accurate communication of offspring need is evolutionarily unstable as offspring will be selected to demand extra resources. Models of parent-offspring conflict have shown that an evolutionarily stable equilibrium can exist at which an offspring solicits resources in a way that reduces its fitness, and a parent provides extra resources to prevent further expensive solicitation^{6–11}. I present an alternative explanation for costly solicitation by showing that the level of offspring solicitation can be a true reflection of offspring need as long as solicitation is costly and the benefits of extra resources increase with need. My analysis suggests that the parent normally allocates resources using accurate information about the condition of the young. The requirement that the signalling system is costly is a direct consequence of the potential for parent-offspring conflict.

Current explanations of costly solicitation are based on models that assume fixed behaviours by either, or both, the parent and the young: parents respond to increased solicitation by providing more resources, and young respond to the delivery of more resources by reduced solicitation^{6–11}. But it is unclear whether the conclusions of these models are robust if the fixed responses of parent and young are themselves allowed to evolve¹². An alternative approach is suggested by recent advances in the theory of biological signals^{13,14}. Although some signals may be physiologically constrained to be unfalsifiable, most have the potential to give false information. For the latter type of biological signals to be evolutionarily stable, they must be costly for the signaller to produce^{15–17}. Recently, Grafen¹⁴ has developed a general framework to study the evolution of such signals and has analysed how animals may signal their quality, for example to potential mates. Using this approach, I show here that the presence of costly solicitation can be explained as a biological signal of need, and also argue that the parent makes decisions on resource allocation based on accurate information about the requirements of the young.

Consider an animal that rears a single offspring per year. Assume that young vary in condition and that a parent is selected to give more resources to young in poor condition. (If the young are in very poor condition, the parent may be selected to cease feeding completely, but here I consider only more minor variation in condition.) The fitness of an offspring ($f(y, x, c)$) depends on its condition (c), the level at which it solicits resources from the parent (x) and the amount of resource received from the parent (y). Offspring fitness rises monotonically with increased resources, though at a decelerating rate. Signalling is expensive so offspring fitness declines (it is assumed monotonically) as x rises. The amount of resource supplied to the young affects the future reproductive success of the parent. For example, a parent that supplies a large amount of food to its offspring may reduce its own condition and lower its probability of survival to the next breeding season. Thus the future fitness of the parent ($g(y)$) decreases (again it is assumed monotonically) as parental investment in the current offspring increases (y). Finally, it is assumed that the amount of resources obtained by the young is some function of the level of display, $y = S(x)$. This function is unknown and is determined from the evolutionarily stable strategy (ESS) conditions.

An ESS¹⁸ exists if neither offspring nor parents gain by a unilateral change in behaviour. I show here that the following strategy pair can be an ESS: (1) the young signal at a rate that is strictly determined by their condition and that increases monotonically as condition worsens; (2) the parent allocates resources to young using the level of display as an accurate indicator of the offspring's condition.

Let x^* and y^* be the ESS values of x and y conditional on c . The ESS can be defined as the point at which an arbitrarily

small increase in begging leads to no net change in inclusive fitness. Suppose that the offspring increases begging from x^* to $x^* + \delta$. The amount of resources obtained by the young increase from y^* to $y^* + S'(x^*)\delta$ where the prime indicates a derivative. The change in behaviour has three effects on inclusive fitness: (1) own fitness increases as the young has more resources; (2) own fitness decreases as increased displaying is costly, and (3) inclusive fitness decreases as increased resource provisioning by the parent reduces its survival. To define the ESS, the net change in inclusive fitness is calculated as a function of δ , and then the limit taken as $\delta \rightarrow 0$,

$$S'(x^*)[f_y(y^*, x^*, c) + r g_y(y^*)] + f_x(y^*, x^*, c) = 0 \quad (1)$$

where, for example, $f_y(y^*, x^*, c)$ is the derivative of $f(y, x, c)$ with respect to y evaluated at the ESS, and r is the coefficient of relatedness of the young to future siblings. Equation (1) was obtained by applying a form of Hamilton's rule. Problems may occur in using this rule to study parent-offspring conflict as changes in fitness are often nonadditive¹⁹. These problems do not arise here because of the assumption of a brood size of one and because approximate additivity obtains in the limit as $\delta \rightarrow 0$ (the marginal Hamilton's rule^{20,21}).

Now consider the behaviour of the parent. The fitness of the parent can be written as proportional to the sum of its reproductive success in the present breeding season plus that in the future: $f(y^*, x^*, c) + g(y^*)$. The ESS level of provisioning (y^*), assuming the parent can correctly assess the condition of its present young, is obtained by maximizing total fitness with respect to y and is given by

$$f_y(y^*, x^*, c) + g_y(y^*) = 0 \quad (2)$$

(Inspection of the second order conditions of equations (1, 2) is necessary to check that the stationary points are maxima and not minima.) To solve equations (1, 2) for the ESS values of solicitation and parental investment as a function of condition, an initial value is required to obtain the exact solution of the differential equation in equation (1). Young in maximum condition ($c_{\max}$) should not solicit ($x = 0$): if the parent uses the level of solicitation to recognize true condition, offspring in top condition can obtain no benefit from displaying. Thus

$$\{y, x, c\} = \{y^*, 0, c_{\max}\} \quad (3)$$

with y^* defined by equation (2), is an ESS solution.

An example of a solution of equations (1-3) is shown in Fig. 1. Offspring in top condition do not display; as the condition of the young deteriorates, both the level of display and the amount of parental investment increases. Fig. 1 also shows the fitness of an offspring as a function of condition: despite the increased parental investment, offspring in poorer condition

have lower fitness. Note that variation in condition is essential for solicitation to be selected: if all offspring are of the same condition then the line of ESSs in Fig. 1 reduces to a point with $x = 0$. By the same argument, greater overall solicitation will be found in circumstances where offspring needs are particularly variable. This may occur, for example, towards the end of the period of parental care when individuals are beginning to fend for themselves. An increase in the level of costly solicitation at this time may be an alternative explanation of 'weaning conflict'.

A mathematical caveat must be stated about the solution of equations (1-3); only local (not global) stability has been demonstrated. The use of phenotypic models also assumes that the predicted solution can be genetically supported. Phenotypic models of parent-offspring conflict may be misleading if the optimal parental strategy depends on the individual's own treatment by its parents. The results presented here apply when parental investment affects viability, but may be modified when fecundity is affected. The analysis can be extended to the case of broods larger than one and to cases where competition is between members of the same brood, rather than between an offspring and future, as yet unborn, siblings. Another complication arises when the costs of solicitation vary between offspring with the same need. The ESS for offspring for whom solicitation is relatively cheap is to misrepresent their needs and to obtain more resources, whereas offspring for whom solicitation is relatively expensive will solicit less and obtain reduced resources. Such behaviour will lead to a resource division that departs from the parental optimum, though the overall reduction in parental fitness is unlikely to be substantial. It has recently been argued that there may be costs to the accurate perception of an honest signal²²; the effects of such costs have yet to be explored.

The model developed here can be compared to Grafen's model¹⁴ of signalling between nonrelatives. In Grafen's model, individuals differ in quality and gain from being perceived as good quality individuals. Signalling of quality can be evolutionarily stable, as long as the costs of signalling are less for better quality individuals. Here, individuals signal need and the costs of signalling do not necessarily differ among individuals. Evolutionary stability in this case requires both that the benefits of costly signalling vary with need, and that the receiver and the signaller are related. Johnstone and Grafen²³ and Maynard Smith²⁴ have analysed a model of altruism (the 'Sir Philip Sidney' game) in which one individual decides whether to give a resource item to another on the basis of a signal of need. Altruism is an ESS if three conditions are met: the two individuals are related, signalling is costly and the benefits of signalling vary with need.

Previous explanations of costly solicitation have emphasized the role of the manipulation of parental behaviour by the offspring; largely because it was unclear how honest signalling

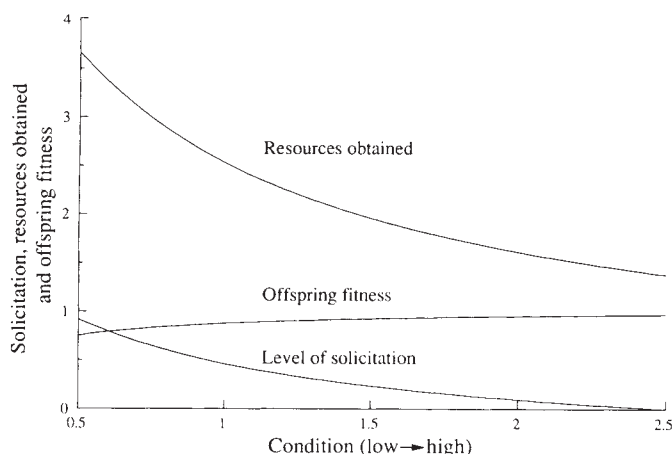


FIG. 1 The ESS level of solicitation by an offspring, the ESS level of resource apportionment by a parent and offspring fitness, as a function of the condition of the young. To solve equations (1-3), the assumption was made that offspring fitness consisted of two additive components: $f(y, c, x) = u(y, c) - v(x)$ where $u(y, c)$ is a function of the resources obtained and condition, and $v(x)$ is a function of the level of solicitation. I have thus assumed that the costs of solicitation are independent of both condition and of the resources obtained (this allows equation (2) to be solved noniteratively). Let $u(y, c) = U(1 - \exp(-cy))$ where U is a constant. Offspring fitness increases with extra resources gained though with diminishing returns and asymptoting at U . The effect of lowered condition is to reduce the rate at which the asymptote is attained. Parents are selected to invest more resources in young of poor condition. I assume that the costs of solicitation are linear: $v(x) = Vx$ where V is a constant, and also that provision of extra resources causes a linear reduction in the parent's future reproductive success: $g(y) = G - \gamma y$ where G and γ are constants. Finally, I assume that for the best quality young, $c = 2.5$. Substitution of these fitness functions into equations (1-3) lead to the following expressions for optimal parental investment and solicitation: $y^* = 1/c \ln(cU/\gamma)$, $x^* = (y^* - y_{c=\max}^*)(\gamma(1-r)/V)$. The following parameter values were used to draw the figure: $U = 1$, $\gamma = 0.08$ and $V = 0.1$.

might be evolutionarily stable. Although offspring manipulation may still be involved in parental care, the model developed here suggests that it is not essential. In the absence of manipulation, do the parents always win the conflict, as suggested by Alexander²⁵? In one sense no; the potential for parent-offspring conflict necessitates a costly signalling system that reduces the fitness

of parent and young. But in another sense the parent does win. The allocation of parental investment is made using accurate information about the condition of the young. The model suggests that chicks beg and young mammals bleat and cry, not to manipulate their parents' behaviour, but as a genuine signal of their need for parental care. □

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Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes

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MARFAN syndrome (MFS), one of the most common genetic disorders of connective tissue, is characterized by skeletal, cardiovascular and ocular abnormalities¹. The incidence of the disease is about 1 in 20,000, with life expectancy severely reduced because of cardiovascular complications. As the underlying defect is unknown, MFS diagnosis is based solely on clinical criteria. Certain phenotypic features of MFS are also shared by other conditions, which may be genetically distinct entities although part of a clinical continuum. Immunohistochemical studies have implicated fibrillin, a major component of elastin-associated microfibrils², in MFS aetiology^{3,4}. Genetic linkage analysis with random probes has independently localized the MFS locus to chromosome 15 (refs 5–7). Here we report that these two experimental approaches converge with the cloning and mapping of the fibrillin gene to chromosome 15q15–21, and with the establishment of linkage to MFS. We also isolated a second fibrillin gene and mapped it to chromosome 5q23–31. We linked this novel gene to a condition, congenital contractural arachnodactyly, that shares some of the features of MFS¹. Thus, the cosegregation of two related genes with two related syndromes implies that fibrillin mutations are likely to be responsible for different MFS phenotypes.

A monoclonal antibody³ was used to isolate and purify a cyanogen bromide-derived peptide fragment of fibrillin. After partial trypsin proteolysis, resultant peptides were further purified and subjected to microsequencing. On the basis of these data, appropriate combinations of sense and antisense oligonucleotides were used to amplify by polymerase chain reaction (PCR) the fibrillin sequences from fibroblast- or lymphoblast-derived complementary DNAs. One of the primer combinations gave rise to a distinct 220-base pair (bp) PCR product found only in the fibroblast samples. This product was used to isolate a 1.6-kilobase (kb) cDNA (MF-13) whose open reading frame includes 19 of 20 residues previously derived from protein microsequencing (Fig. 1a). Moreover, MF-13 hybridizes to a messenger RNA whose length (10 kb) is consistent with the size of the fibrillin translation product (relative molecular mass 350,000; *M_r* 350K) (ref. 8) (data not shown). Finally, the published amino-acid composition of fibrillin and that derived from MF-13 are nearly identical⁹. Some distinctive structural features of fibrillin are apparent even in the partial sequence encoded by MF-13. The most evident is the repetitive nature of the polypeptide, which is composed of several cysteine-rich, epidermal growth factor (EGF)-like repeats¹⁰. An additional feature is the relatively high content of acidic amino acids accounting for nearly 15% of the residues (Fig. 1).

To isolate the fibrillin gene, a λ phage genomic library was screened with MF-13 under mildly stringent conditions (see legend to Fig. 1). Several positive clones were identified and segregated into three distinct groups by restriction mapping, selective sequencing and chromosomal localization.

Three exons, one of 129 bp and two of 126 bp, were identified in the first set of overlapping clones (Fig. 1c). They correspond to an internal 381-bp sequence of MF-13 (Fig. 1a). *In situ* hybridization mapped the corresponding locus to 15q15–21 (Fig. 2a); hereafter, this fibrillin gene will be termed Fib 15. As the chromosomal location of Fib 15 is roughly that independently established for the MFS locus using random probes^{5–7} we investigated whether Fib 15 is linked to MFS. To this end, two polymorphic DNA markers (Fig. 1c) were used to genotype several multi-generation MFS families, three of which were found to be informative (Fig. 3a). No recombinations were observed and a combined logarithm of the odds (lod) score of 7.56 at the maximum likelihood of recombination (Θ) = 0.00 was obtained. This demonstrated tight genetic linkage between Fib 15 and MFS (Fig. 4).

In the second group of genomic clones, a common 10-kb *EcoRI* fragment hybridized with MF-13 under low stringency conditions (see legend to Fig. 1). Further mapping of this subclone identified three exons encoding EGF-like sequences

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Deceased.

a	GTT	TTA	GTG	ACA	GTT	GTA	TTT	ATT	TTT	TTA	AGT	TAC	AAT	AAA	ATG	CTA	TCA	51
	L	V	T	V	V	F	I	F	L	S	Y	N	K	M	L	S		
	AGT	CCT	TGC	ATT	AAT	GGA	GTC	GTG	AAG	AAC	GCA	GGC	TCT	TTT	ATT	TGT	102	
	S	P	C	I	N	G	V	C	K	N	S	P	A	G	T	I	C	
	GAA	TGT	TCT	TCT	GAA	AGT	ACT	TTG	GAT	CCA	ACA	AAA	ACC	ATC	TGC	ATA	153	
	A	C	S	S	E	S	T	L	D	P	T	K	T	I	C	I	E	
	AOC	ATC	AGG	GGC	ACT	TGC	TGG	CAG	TCT	GTT	ATT	GGG	GCA	TGT	GAG	ATC	204	
	I	K	G	T	C	T	W	Q	T	V	I	D	G	R	C	E	I	
	AAC	ATC	AAT	GGA	GCC	ACC	TTC	AAG	TCC	CAG	TGC	TGC	TCC	CTC	GGT	GCT	255	
	N	I	N	G	A	T	L	K	S	Q	C	C	S	S	L	G	A	
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	A	W	G	S	P	C	T	L	C	Q	V	D	P	I	C	G	K	
	GGG	TAC	GTA	AGA	ATT	AAA	GCA	ACA	TGT	GAA	GAT	ATA	GAT	GAA	TGT	GAA	357	
	G	Y	C	S	A	I	K	G	T	Q	C	E	D	I	D	E	C	
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	V	F	P	F	G	V	C	K	N	G	L	C	V	N	T	R	G	
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	F	K	C	Q	C	P	S	G	M	T	L	D	A	T	G	R	I	
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	C	L	D	I	R	L	E	T	C	F	L	R	Y	E	D	E	E	
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	C	T	L	P	I	A	G	R	H	R	M	D	A	C	C	C	S	
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	V	G	A	A	W	G	T	E	E	C	E	C	P	P	M	R	N	
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	T	P	E	Y	E	E	L	C	P	R	G	P	G	F	A	T	K	
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	K	C	R	C	D	S	G	F	A	L	D	S	E	E	R	N	C	
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	T	D	I	D	E	C	R	I	S	P	A	D	L	T	G	C	G	
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	C	V	N	T	P	G	D	F	E	C	K	K	D	E	G	Y	E	
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	S	G	F	M	M	K	N	C	M	D	I	D	E	C	Q	R		

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53	N	E	C	A	T	A	L	D	P	D	I	C	A	N	G	I	C
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55	L	R	G	S	T	Y	R	C	N	C	N	S	G	Y	E	P	D
56	TCT	GGA	AGA	AAC	TGT	ATC	ATT	GAT	ACA	TGT	TTA	GTA	AAC	AGA	CTG	CTT	S
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58	TGT	GAT	AAC	GGA	TTG	TGC	AGA	AAC	ACG	ACA	GGA	AGT	TAC	AGC	TGT	AGC	TGC
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67	I	C	I	D	S	L	K	G	C	T	W	L	N	I	Q	D	S
68	CGC	TGT	GAG	GTG	AAT	ATT	AAT	GAC	ACT	CTG	AAA	TCT	GAA	TGC	TGT	GCG	T
69	C	E	V	N	I	N	G	A	C	T	L	G	K	S	E	C	A
70	ACC	CTC	GGA	GCC	GCG	TGG	GCG	ACC	TCC	TGT	CAG	GGT	GAA	CTA	GAT	ACA	
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75	N	E	C	A	E	V	F	P	G	V	C	P	N	G	R	C	V
76	AGT	AAG	GSA	TCT	TTT	CAT	GTC	TGC	CGT	ACA	GGC	CTT	ACG	TTG	GAT	GGG	S
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78	ACT	GCG	CGT	GTA	TGT	TTG	GAT	ATT	CGC	ATG	GAG	CAG	TGT	TAC	TTG	AAG	TGG
79	T	G	R	V	C	L	D	I	R	M	E	Q	C	Y	L	K	W
80	GAT	GAA	GAT	GAA	TGC	ATC	CAC	CCC	TTT	CGT	GGA	AAG	TTT	CGC	ATG	GAT	GCC
81	D	E	D	E	C	I	H	P	V	P	C	G	K	F	R	M	D
82	TGC	TGT	GCT	GCT	GGG	GCG	GCT	TGG	GCG	ACC	TGT	GAG	GAT	GAG	GAG	TGC	CCC
83	C	C	C	A	V	G	A	W	T	E	E	C	E	C	E	C	P
84	AAA	CGT	GGC	ACG	GAA	TAC	GAG	ACA	CTG	TGC	CCC	CGC	GGG	CGT	GGC	TTT	K
85	K	P	G	T	K	E	Y	E	T	L	C	P	T	A	G	A	G
86	GCT	AAC	GGA	GGG	GAT	GTT	CTT	CGC	GGG	CGA	CTT	TAC	AAA	GAC	ATC	AAT	A
87	A	N	R	G	D	V	L	T	G	R	P	F	A	Y	K	D	I
88	GAA	TGC	AAA	GGA	TTT	CGT	TGC	TGC	ACT	TAT	GGG	AAG</					

FIG. 1 *a, b*, Nucleotide sequence and single-letter amino-acid translation (below) of human fibrillin cDNAs MF-13 (*a*) and MF-23 (*b*). Numbering begins with the first nucleotide of each insert. *a*, Boxed residues correspond to the 20 amino-acid peptide derived from protein microsequencing. The translated nucleotide sequence differs from the peptide sequence by one residue, the encircled serine in place of aspartic acid. The underlined sequences demarcate the ends of the 220-bp PCR-amplified cDNA product (see methods). *b*, The underlined sequence identifies the oligonucleotide used in the cDNA library screening. The vertical bars delineate three exons identified in the respective genes (Fib 15 and Fib 5, *c*). Each exon encodes an EGF-like, cysteine-rich domain. *c*, Partial restriction map of the genomic fragments found in the Fib 15 and Fib 5 loci with their sizes (in kb). *EcoRI*(E), *BamHI*(B) and *SphI*(S) sites are indicated. The relative positions of the three exons encoding Fib 15 and Fib 5 sequences are shown to scale below, along with their sizes (in bp). Also indicated are the relative locations of two repetitive sequences (▽), a Fib 15-specific (TAAAA)_n and a Fib 5-specific (GT)_n repetitive block, used in the linkage experiments.

METHODS. Microsequencing of fibrillin peptides produced three fibrillin-specific sequences: a 20 amino-acid peptide, IPSLCTHGKCRXTIGDFKCR; a 16 amino-acid peptide, TSAGSDINEXALDPDI; an 11 amino-acid peptide, CLDINECEPDA. One inosine-containing degenerate primer pair derived from the 20 and 11 amino-acid peptide sequences (5'-ACIATIGGIGAT(T/C)TTT(T/C)AAG(A/ATG-3' and 5'-GGT(G/C)TC(A/G)CAITCA(G/TTIAT(A/G)TC-3', respectively) consistently produced a fibroblast-specific 220-bp PCR-amplified cDNA fragment (a). The primers annealed and amplified a region different from that demarcated by the 20 and 11 amino-acid sequences due to the repetitive

nature of the EGF-like, cysteine-rich domains. The sequence of the 11 amino-acid peptide is not encoded by MF-13. Double-stranded cDNA was synthesized by random-priming of 5 µg of total RNA using a commercial kit (Amersham). PCR amplification conditions were as previously described¹³, except for annealing at 55 °C for 2.5 min and extension at 72 °C for 1.5 min. Screening of cDNA (Clontech) and genomic (Clontech and Stratagene) λ phage libraries using cDNA or oligonucleotide probes was done according to standard conditions¹⁴. Low-stringency screening of the genomic library with MF-13 was done with standard hybridization conditions¹⁴ but with termination of washing after 0.5 × SSC medium at 42 °C for 30 min. Sequencing was by the dideoxynucleotide chain termination method¹⁵ (Sequenase, USB).

FIG. 2 Chromosomal mapping of the Fib 15 (a) and Fib 5 (b) genes. a, b, The left top shows a partial view of a human metaphase spread with arrow-heads indicating silver grains on Giemsa-stained chromosomes, after autoradiography. Below is the same spread subsequently identified by R-banding. On the right is an idiogram of the positive chromosomal localization illustrating the distribution of the labelled sites for each of the fibrillin probes. METHODS. *In situ* hybridization was done as previously described¹⁶ using two ³H-labelled probes: a 1-kb fragment spanning the three-exon region of Fib 15 (Fig. 1a, c), and a 500-bp *Pst*I fragment lying immediately 3' to the Fib 5 variable number of dinucleotide repeats (Fig. 1c). For each *in situ* hybridization, 200 metaphase cells were examined. Using the Fib 15 probe, 15% of the silver grains were localized on chromosome 15, and 68.2% of them mapped to q15–21. Using the Fib 5 probe, 14.4% of the silver grain were localized on chromosome 5, and 70% of them were mapped to q23–31.

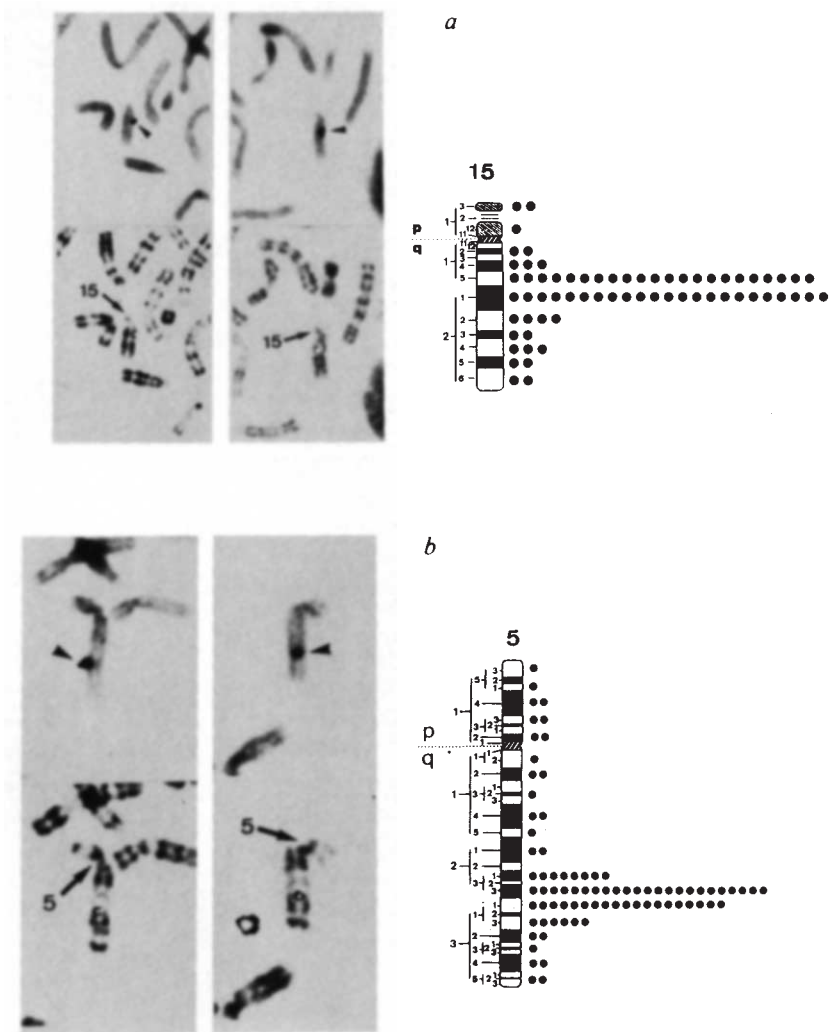


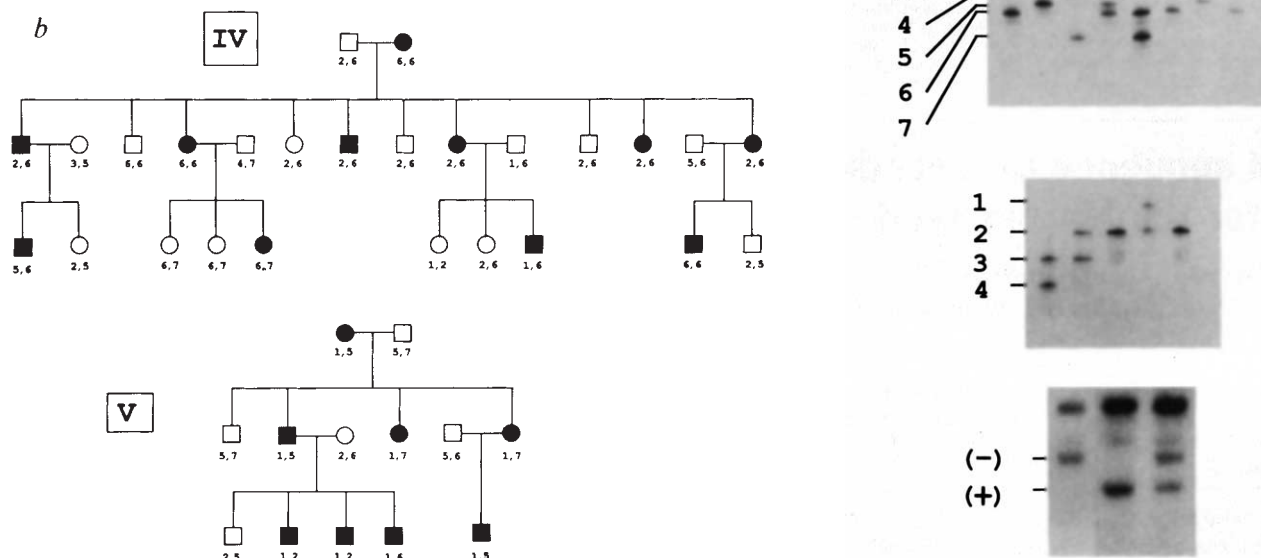
FIG. 4 Summary of the linkage data for MFS and CCA families and indication of the most salient clinical manifestations in the two sets of families. Lod scores were calculated with the LIPED¹⁸ and LINKAGE¹⁹ programs with data preparation by the dBLINK package (M.S., unpublished). Marker allele frequencies were assumed to be equal. MFS linkage to Fib 15 was calculated with haplotype data derived from the *Taq*I restriction fragment length polymorphism and the (TAAAA)_n repeat. The three MFS families were included in a previous linkage study using chromosome 15 anonymous probes⁷. MFS manifestations include cardiovascular abnormalities (aortic regurgitation, aortic root dilatation/dissection and mitral valve prolapse), ocular problems (ectopia lentis and myopia), and skeletal deformities (kyphoscoliosis, pectus deformities, tall stature and arachnodactyly). CCA manifestations include flexion contractures and abnormal pinnae. Major cardiovascular or ocular abnormalities were not observed in the CCA patients.

Linkage of Marfan Syndrome to Fib 15 Haplotypes					
Family	Recombination Fraction				
	0.000	0.100	0.200	0.300	0.400
I	3.783	3.163	2.465	1.741	0.917
II	2.056	1.663	1.248	0.829	0.417
III	1.720	1.375	1.005	0.608	0.212
Totals	7.559	6.201	4.658	3.178	1.546
$Z_{max} = 7.559, \theta_{max} = 0.00, CI: 0.0-0.08$					
Clinical Manifestation of MFS Families					
Family	Skeletal	Cardiovascular	Ocular		
I	+	+	+		
II	+	+	+		
III	++	+	+		

Linkage of Congenital Contractural Arachnodactyly to Fib 5 VNDR					
Family	Recombination Fraction				
	0.000	0.100	0.200	0.300	0.400
IV	2.107	1.787	1.429	1.023	0.554
V	2.408	1.996	1.538	1.029	0.490
Totals	4.515	3.783	2.967	2.052	1.044
$Z_{max} = 4.515, \theta_{max} = 0.00, CI: 0.0-0.14$					
Clinical Manifestation of CCA Families					
Family	MFS Hapitus	Abnormal Pinnae	Contracture	Cardiovascular	Ocular
IV	-	+	+	-	-
V	+	+	+	-	-

FIG. 3 Segregation of fibrillin polymorphic DNA markers. Available genotypes of Fib 15 markers (a) in MFS (I, II, III) and Fib 5 marker (b) in CCA (IV, V) families. c, Autoradiograms of electrophoretic migration of the PCR-amplified repetitive markers and *TaqI* restriction fragment length polymorphism. From top to bottom, they are: a (GT)_n VNDR sequence (alleles 1–7, 110–155 bp) for Fib 5, and a (TAAA)_n sequence (alleles 1–4, 150–180 bp) and a *TaqI* RFLP (alleles (+) 5 kb, and (–) 6 kb), for Fib 15.

METHODS. RFLP analysis for the Fib 15 locus was by Southern blot hybridization¹⁴ to MF-13 of *TaqI*-digested DNA. Genotyping of repetitive sequences was by PCR amplification of genomic DNA with flanking oligonucleotide primers (Fib 5: 5'-AAGGTGTTCTTTGCATGTTCCAC-3' and 5'-GTAATGTGTTCTATCTAGTTCAACG-3' and Fib 15: 5'-CCTGGCTACCATTCACCTCC-3' and 5'-GAGTACATAGAGTGTGTTTGGG-3'). Amplification conditions have been previously described¹⁷ except for the annealing temperatures (Fib 5 primers were annealed at 55 °C for 2.5 min, whereas Fib 15 primers were annealed at 50 °C for 2.5 min), and the phosphorylation of one oligonucleotide primer in each reaction with γ -P³² ATP (3,000 Ci mmol⁻¹) and T4 polynucleotide kinase (Promega)¹⁴. 1 μ l of the denatured PCR reaction product was electrophoresed in a 7 M urea/5% polyacrylamide gel.



similar to those of Fib 15 (Fig. 1a, c). This gene was termed Fib 5 after its chromosomal localization to 5q23–31 (Fig. 2b). An oligonucleotide derived from a divergent sequence of the Fib 5 exons was used to isolate a 2.8-kb cDNA (MF-23). The putative amino-acid translation of MF-23 demonstrated that this gene is structurally related to MF-13. Like Fib 15, Fib 5 is characterized by repeated cysteine-rich, EGF-like motifs and by a relatively high content of acidic amino acids (Fig. 1b). In addition, MF-23 and MF-13 hybridize to transcripts of the same size (data not shown). Distinct segments of the two fibrillin molecules are encoded by three exons having identical organi-

zation (Fig. 1). It is, therefore, conceivable that the fibrillin genes may be composed of multiple repeats of similarly sized exons coding for EGF-like motifs.

These findings provided the first demonstration of the genetic heterogeneity of fibrillins. As several conditions share some of the phenotypic features of MFS, we investigated whether Fib 5 was linked to an MFS-related disorder. These conditions include the familial forms of arterial dissection, mitral valve prolapse and annuloaortic ectasia¹, as well as the marfanoid hypermobility syndrome and congenital contractural arachnodactyly (CCA), once thought to be phenotypic subtypes of MFS^{11,12}.

Genotyping of two CCA kindreds established linkage between Fib 5 and this MFS-related phenotype with a maximal combined lod score of 4.5 at $\Theta = 0.00$ (Fig. 3b, and Fig. 4).

Linkage between a candidate gene and a disease locus, although highly suggestive, does not constitute final proof of causal relationship. In this case, however, linkage between two structurally related genes and two phenotypically related disorders gives strong support to this causal association. Hence, mutations in different fibrillin genes may be the cause of distinct phenotypes belonging to the clinical spectrum of MFSs. In agreement with this, preliminary data indicate that the third group of genomic clones isolated with MF-13 belongs to an additional fibrillin gene residing on chromosome 17. $\square$

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Partial sequence of a candidate gene for the Marfan syndrome

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FIBRILLIN is a large (relative molecular mass 350,000) glycoprotein which can be isolated from fibroblast cell cultures and is a component of the microfibrils that are ubiquitous in the connective tissue space¹. The microfibrils of the suspensory ligament of the lens as well as the elastic fibre microfibrils of the blood vessel wall are composed of fibrillin. The ocular and cardiovascular manifestations of the Marfan syndrome are consistent with a defect in the gene coding for a structural constituent of these connective tissues. Immunohistological experiments have recently implicated fibrillin microfibrils in the pathogenesis of the Marfan syndrome². Genetic linkage data^{3,4} localizing the Marfan gene to chromosome 15 and the *in situ* hybridization of fibrillin complementary DNA

to 15q21.1 (ref. 5) together support fibrillin as a candidate Marfan gene. As a first step towards investigating the function of fibrillin in the architecture and development of connective tissues and its relationship to the Marfan syndrome, we report the cloning and partial sequencing of fibrillin cDNA.

Fibrillin cDNA clones hybridized to a single messenger RNA of about 10 kilobases (kb), which is consistent with the relative molecular mass of the glycosylated protein. Twelve different clones, covering 6.9 kb of the estimated total 10 kb transcript, have been isolated. The nucleotide sequence and the amino-acid sequence deduced from the fibrillin cDNA clones is shown in Fig. 1. There is an open reading frame of 5,920 base pairs (bp), followed by a termination codon and 916 bp of 3' untranslated region. A potential polyadenylation consensus sequence, AATAAA, beginning at position 6,809, is located 23 bp upstream from the poly(A) tail.

The deduced amino-acid sequence contains 394 amino-acid residues which have been confirmed by comparison with the known sequences of 23 fibrillin peptides, including the amino-terminal sequence for PF2, a fragment of fibrillin obtained from pepsin digests of human amnion⁶. (There is an error in the N-terminal sequence of PF2 given in ref. 6: position 4, reported as Ile, should be Gly.) The amino terminus of PF2 begins at amino acid 861 (nucleotide 2,581) and other PF2 sequences are found in the adjacent sequence through to amino acid 1,247 (nucleotide 3,741). PF1 amino-acid sequences have been located in the region 5' to PF2 sequences, but the amino-terminal sequence of PF1 (ref. 6) has not yet been identified by cDNA cloning. There are 14 potential glycosylation sites in the stretch of 1,973 amino acids.

Dotplot analysis of the amino-acid sequence compared with itself to look for internal sequence homology revealed two distinct sequence motifs, a 6-cysteine repeat and an 8-cysteine repeat, and the carboxy terminus of 184 amino acids, which has no homology to the rest of the protein.

Comparison of fibrillin sequences with nucleic acid and protein databases reveals significant homology between 34 6-cysteine repeats in fibrillin and two sets of tandem repeats between positions 314 and 477 and between 741 and 952 in the precursor molecule of human epidermal growth factor (EGF) (Fig. 2a). Fibrillin sequences show more homology to sequences in precursor EGF than to the bioactive EGF peptide. There is also significant homology to the EGF-like repeats found in genes such as *Notch* in *Drosophila*⁷ and *lin-12* in *Caenorhabditis elegans*⁸. In addition, the fibrillin EGF-like repeats contain the

FIG. 1 Partial cDNA and deduced amino-acid sequences of human fibrillin. The composite cDNA sequence assembled from overlapping cDNA clones is shown with the deduced amino-acid sequence. Nucleotides and corresponding amino acids are numbered starting with the first base of the reported sequence. There are 6,900 nucleotides and 1,973 amino-acid residues, ending with the termination codon at nucleotides 5,920-5,922. The termination codon is followed by 916 nucleotides of 3' untranslated region and 62 bp of poly(A) tail. Regions corresponding to sequenced fibrillin peptides are underlined.

METHODS. A λ gt11 oligo(dT) primed human placental cDNA library (Clontech) was screened with a mixed pool of oligonucleotides representing all coding possibilities for the first 20 bp of a fibrillin peptide sequence, CEDIDE, by base composition-independent hybridization¹³. Positive clones were screened by northern blot analysis for hybridization to messenger RNA of an appropriate size. A total of 12 overlapping cDNA clones were analysed. Double- and/or single-stranded DNA from 6 overlapping clones covering 6.9 kb was completely sequenced by the dideoxy chain termination method using Sequenase V2.0 (US Biochemical). In addition, a minimum of 300 bp from each end of all the remaining clones was sequenced. All sequences were confirmed with independent overlapping clones and/or the opposite strand. Sequence fragments were assembled and translated using the Sequence Analysis Software Package of the Genetics Computer Group. Peptide sequences were determined from tryptic, V8 protease, and cyanogen bromide peptides of pepsin fragments PF1 and PF2 (ref. 6). Digests were separated using reversed-phase HPLC and sequenced as described¹⁵.

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[illegible]

a

EGF Precursor	314	EQKL CKLRKGN	CSSTV	CGQDLQSHL	CMC	AEGYALSRDRKY	CE	355
	356	DVNE CAFWNHG	CTLG	CKNTPGSY	CTC	PVGFLVLLPDGKR	CH	396
	397	QLVS CERNVSE	CSHD	CVLTSEGFL	CFC	PEGSVLERDGT	CS	437
	438	G CSSPDNGG	CSQL	CVPLSPVSW	CDC	FPQYDLQLDEKS	CA	477
	741	GADP CLYQNGG	CEHI	CKKRLGTAW	CSC	REGFMKASDGKT	CL	781
	831	DQDD CAPVG	CSMYAR	CISEGEDAT	CQC	LKGFAGDGKL	CS	869
	870	DIDE CEMGVFV	CPPASSK	CINTEGGYV	CRC	SEGYYQGDGIH	CL	911
	912	DIDE CQLGVHS	CGENAS	CTNTEGGYT	CMC	AGRLSEFGLI	CP	952
Bioactive EGF	971	NSDSE CPLSHDGY	CLHDGV	CMYIEALDKYA	CNC	VVGYIGER	CQYRDLKWWE	1023

Fibrillin	12	DIDE CEVFPV	CKNGL	CVNTRGSFK	CQC	PSGMTILDATGRI	CL	53
	130	DINE CKMIPSL	CTHGG	CRNTIGSFK	CRC	DSGFALDSEERN	CT	171
	172	DIDE CRISPD	CGRGQ	CVNTPGDFE	CKC	DEGYESGFMMKKN	CM	214
	215	DIDE CQRDPL	CRGGV	CHNTEGSYR	CEC	PPGHQLSPNISA	CI	256
	257	DINE CELSAHL	CPNGR	CVNLIGKYQ	CAC	NPGYHSTPDRLF	CV	288
	289	DIDE CSIMNGG	CETF	CTNSEGSYE	CSC	QPGFALMDQRS	CT	339
	340	DIDE CEDNPN	CDGGQ	CTNIPGEYR	CLC	YDGFMAEDMKT	CV	381
	382	DVNE CDLNPN	CLSGT	CENTGSGFI	CHC	DMGYSGKKGKTG	CT	423
	424	DINE CEIGAHN	CGKHAV	CTNTAGSFK	CSC	SPGWIGDGIK	CT	464
	465	DIDE CSNGTHM	CSQHAD	CKNTMGSYR	CLC	KEGYTGDGFT	CT	505
	506	DIDE CSENHNL	CGNGQ	CLNAPGGYR	CEC	DMGFVPSADGKA	CE	547
	548	DIDE CSLPNI	CVFGT	CHNLPGLFR	CEC	EIGYELDRSGGN	CT	588
	589	DVNE CLDPTT	CISGN	CVNTPGSYI	CDC	PPDFELNPTRVG	CV	629
	708	DIDE CQELPGL	CQGGK	CINTGSGFQ	CRC	PTGYLLNEDTRV	CD	749
	750	DVNE CETPGI	CGPGT	CYNTVGNIT	CIC	PPDYMQVNGGNN	CM	790
	868	DIDE CREIPGV	CENGW	CINMGVSFR	CEC	PVGFFYNDKLLV	CE	909
	910	DIDE CQNGPV	CQRNAE	CINTAGSYR	CDC	KPGYRFTSTGQ	CM	950
	951	DRNE CQEPNI	CSHGQ	CIDTVGSFY	CLC	HTGFKTNDQTM	CL	992
	993	DINE CERDA	CGNGT	CRNTIGSFN	CRC	NHGFILSHNND	CI	1031
	1032	DVDE CASGNGNL	CRNGQ	CINTVGSFQ	CQC	NEGYEVAPDGR	CV	1074
	1075	DINE CLLEPRK	CAPGT	CQNLDSGYR	CIC	PPGYSLQNEK	CE	1114
	1115	DIDE CVLEPEI	CALGT	CSNTEGSFK	CLC	PEGFSLSSGFR	CQ	1156
	1229	DMDE CKEPDV	CKHQQ	CINTDGSYR	CEC	PPGYTLAGNE	CV	1267
	1268	DTDE CSVGNP	CGNGT	CKNVIGGFE	CTC	EEGFEPGPMNT	CE	1307
	1308	DINE CAQNPL	CAFR	CVNTYGSYE	CKC	PVGYVLREDARM	CK	1348
	1349	DEDE CEEGKHD	CTEKQME	CKNLIGTYM	CIC	PGGYQRFPDGE	CV	1392
	1393	DENE CQTKPGI	CENGR	CLNTRGSYT	CEC	NDGFTAFSPQDE	CL	1434
	1504	DIDE CKVIHDV	CRNGE	CVNDRGSYH	CIC	KTGYTPDITGTS	CV	1545
	1546	DLNE CNQAPKP	CNFI	CKNTEGSYQ	CSC	PKGYILOEDGRS	CK	1586
	1587	DIDE CATKQHN	CQFL	CVNTIGGFT	CKC	PPGFTQHHTS	CI	1625
	1626	DNNE CTSIDNL	CGSKGI	CQNTPGSFT	CEC	QRGFSLDQTGS	CE	1668
	1669	DVDE CEGNHR	CQHG	CQNIIGGYR	CSC	PPGYLQHYQWNG	CV	1708
	1709	DENE CLSAHI	CGGAS	CHNTLGSYK	CMC	PAGFYEQFSGG	CQ	1749
	1750	DINE CGSAQAP	CSYG	CSNTEGGYL	CQC	PPGYFRIGQGH	CV	1789

Fibrillin
Consensus

DIDE C----- C--G- C-NT-GSY- C-C --GY----- C-
N D F D

b

TGF-β1-BP	347	SEKGP CYRLVSSGRQ	CMYPLSVHLTKQL	CCC	SVGKA GPH CEK	CPLPGTAAFEICPGG	MGYTVSGVHRRP	418
	1016	KEEKKECYNLNDASL	CDNVLAPNVTKQE	CCC	TSAGAGWGN	CEIFPCPVLTAEFTMC PKG	KGFVPAGESSEA	1090
	1193	DVYQDLCEWHLSDYV	CSRPLVGKQTTYTE	CCC	LYGEAWGMQ CAL	CPLKDDDYAQLCNIPVTGRRQPYGRDALV		1267
Fibrillin	54	DIRLETCLFRYEDDE	CTLP IAGRHMDA	CCC	SVGAANGTECEE	CPMRNTPYEELCPRG	PGFATKEITNGKPFK	129
	630	DIRSGNICYLDIRPGDNGDTACSNEIGVGVSKAS	CCC	SLGKANGTP CEM	CPAVNTSEYKILCPGG	EGFRPNPITVILE		707
	791	DMRRSLCYRNYADNQT	CDGELLFNMTKQM	CCCSYNIGRAWNKP	CEQ	CPIPSTDEFATLCGSRPGFVIDIYTGLPV		867
	1157	DLRMSYCYAKFEGGK	CSSPKSRNHSKQE	CCC	ALKGEGWQDF CEL	CPTEPDEAFRQICPYG	SGIIVGPDSDAV	1228
	1435	DNREGYCFTEVLQNM	CQIGSSNRNPVTKSECC		DGGRGWGFH CEI	CPFGQTVAFKKLCPHG	RGFMINGA	1503

Fibrillin
Consensus

D-R---CY----- C-----K-- CCC --G-ANG-P CE- CP---T-ET--LCP-G -GF-----T---
F G N D AY I G Q I

c

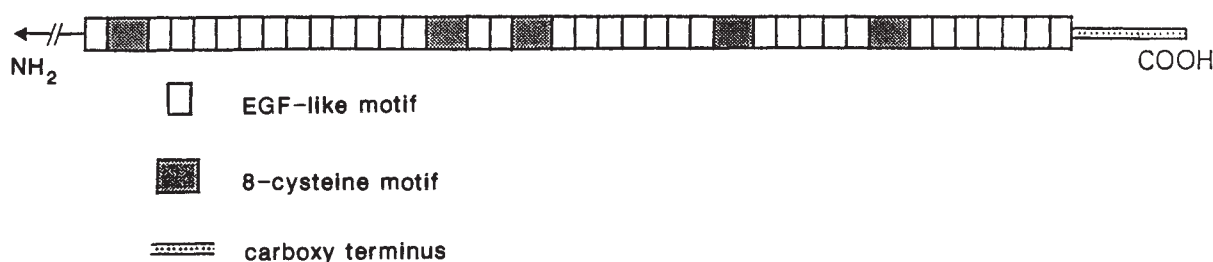


FIG. 2 Homologies between the two types of fibrillin motifs and a, EGF precursor and b, TGF- β 1-binding protein (TGF- β 1-BP). a, The 34 EGF-like repeats in fibrillin are aligned with the nine 6-cysteine repeats from EGF precursor (including the homologous sequence of bioactive EGF). Gaps have been introduced to align the cysteines. The fibrillin repeats are more similar to the repeats of EGF precursor than to mature EGF. A consensus sequence for the fibrillin EGF-like repeats is shown. b, The 8-cysteine motif in fibrillin is compared with three similar repeats found in TGF- β 1 binding protein¹⁰. The consensus sequence for this motif in fibrillin is identified. Five repeats of the 8-cysteine motif interrupt the 34 tandem blocks of EGF-like repeats. A graphic representation of these motifs within the fibrillin gene is shown in c.

METHODS. Homology between fibrillin and EGF was determined by searching the GenEMBL database, release 67 (ref. 16), using TFASTA, and the Protein Identification Resource database, release 28 (ref. 17), with SEARCH. Consensus amino acids are shown if they occur in at least 50% of the repeats. Both amino acids are shown where there are only two choices. Dashes indicate positions where there is no consensus. The consensus sequence for aspartic acid/asparagine hydroxylation is indicated by a bar.

conserved consensus sequence CX(D/N)X₄(F/Y)XCXC (one-letter amino-acid code, X is any residue; Fig. 2a), which has been associated with hydroxylation of asparagine or aspartic acid residues in EGF-like domains of other proteins⁹.

The second motif, containing eight cysteines, is repeated five times in fibrillin. This motif is homologous to three repeats found in transforming growth factor- β 1 (TGF- β 1) binding protein¹⁰ (Fig. 2b), which also contains 16 6-cysteine EGF-like repeats. In fibrillin and TGF- β 1 binding protein, the 8-cysteine repeats intersperse the EGF-like repeats. It will be interesting to determine whether fibrillin and TGF- β 1 binding protein have functional similarities as well as structural homology. Also, fibrillin has one RGD sequence, at positions 643–645, which is in an 8-cysteine motif. Peptides containing the RGD sequence have been implicated in cell attachment activity of extracellular matrix proteins¹¹.

The actual amino-acid composition of monomeric fibrillin¹² and the composition of the 1,973 amino acids deduced from the partial cDNA sequence are consistent. They are both notably rich in cysteine residues, about 33% of which are in the free reactive sulphhydryl form¹². If the 34 EGF-like repeats have an internal disulphide bond pattern similar to EGF, then the 8-cysteine intervening sequences might contain the free cysteines and participate in disulphide bonding. The carboxy terminus is a cysteine-poor region (only two of the 184 amino acid residues are cysteines) which is unusually rich in lysine and arginine residues.

In the accompanying paper¹³, data establishing linkage between the fibrillin locus and the Marfan phenotype are presented. In addition, a mutation has been discovered which results in a single amino-acid substitution in an EGF-like repeat in a patient with the Marfan syndrome¹³. Knowing the nucleotide sequence and primary structure of fibrillin will help us to understand the normal function of fibrillin in connective tissue and how abnormal fibrillin can result in the Marfan phenotype. □

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Marfan syndrome caused by a recurrent *de novo* missense mutation in the fibrillin gene

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MARFAN syndrome is an inherited disorder of connective tissue manifested in the ocular, skeletal and cardiovascular systems. It is inherited as an autosomal dominant with high penetrance, but has great clinical variability¹. Linkage studies have mapped the Marfan locus to chromosome 15q15–21.3 (refs 2, 3). There have been no reports of genetic heterogeneity in the syndrome. Following the identification of fibrillin (a glycoprotein component of the extracellular microfibril⁴), immunohistopathological quantification of the protein in skin and fibroblast culture⁵, and examination of fibrillin synthesis, extracellular transport, and incorporation into the extracellular matrix (D. M. Milewicz, R.E.P., E. S. Crawford and P. H. Byers, manuscript in preparation) have demonstrated abnormalities of fibrillin metabolism in most patients. A portion of the complementary DNA encoding fibrillin has been cloned⁶ and mapped by *in situ* hybridization to chromosome 15 (ref. 7). Here we report that the fibrillin gene is linked

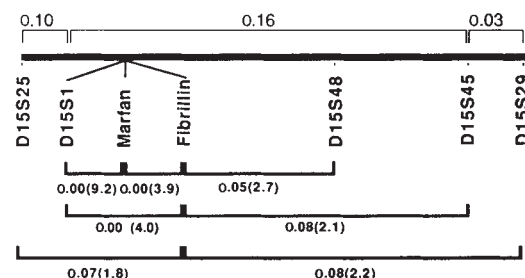


FIG. 1 Summary of two-point linkage relationships between fibrillin polymorphism, Marfan phenotype and five other polymorphic markers on chromosome 15. Map distances (expressed as recombination fractions) and lod scores (in parentheses) are shown for each two-point analysis. Four markers were ordered from centromere to telomere (cen-D15S25-D15S1-D15S45-D15S29-tel) according to a published map¹⁹. Corresponding map distances are shown at the top of the figure. D15S48 has been previously mapped to this region²⁰; odds do not allow precise placement in relation to other markers.

METHODS. Family identification, phenotype assignment, DNA isolation, restriction fragment length polymorphism analysis and linkage analysis were done as previously described³. Fibrillin polymorphism (2 allele system; 4.8-kb uncut allele, 4.3-kb cut allele) was recognized by hybridization of cDNA clone pCLM9 (ref. 6) to *Taq*I-digested genomic DNA.

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to the Marfan phenotype ($\theta = 0.00$; logarithm of the odds (lod) = 3.9) and describe a *de novo* missense mutation in the fibrillin gene in two patients with sporadic disease. We thus implicate fibrillin as the protein defective in patients with the Marfan syndrome.

A *TaqI* restriction-site polymorphism detected by the fibrillin cDNA clone pCLM9 (ref. 6) (4.8-kilobase (kb) and 4.3-kb marker alleles) was identified and used to assess the linkage relationship between the fibrillin locus and the Marfan syndrome phenotype in three multiplex families. The maximum likelihood estimate of recombination ($\hat{\theta}$) was 0.00 for the two informative families (Table 1). In addition, no recombinants were observed between fibrillin and D15S1, the anonymous DNA fragment most closely linked to the Marfan locus³. Results of two-point linkage analysis between fibrillin, the Marfan syndrome phenotype, D15S1 and four other polymorphic chromosome 15 markers (Fig. 1) demonstrate tight linkage between fibrillin and the Marfan syndrome locus with a θ of 0.00 ± 0.12 at a lod score of 3.9 (odds about 8,000:1 favouring linkage at no recombination).

To determine whether fibrillin is defective in patients with Marfan syndrome, we searched for disease-producing mutations in the fibrillin-coding sequence. Initial efforts failed to identify restriction-fragment size alterations by Southern hybridization of fibrillin cDNA probes to genomic DNA from 38 unrelated affected individuals. In the absence of gross gene rearrangements, we sought to identify point mutations. Polymerase chain reaction (PCR)-amplified cDNA, reverse-transcribed from fibroblast total RNA from patients with *de novo* Marfan syndrome, was screened using single-strand conformation polymorphism (SSCP) analysis. An abnormally migrating band was observed in 1 out of 14 patient samples (Fig. 2, lane 3). This patient (E.S.) had severe manifestations of the Marfan syndrome noted in early infancy including increased body length, arachnodactyly, muscular hypotonia, joint laxity and mitral regurgita-

TABLE 1 Linkage of a fibrillin polymorphism and the Marfan syndrome phenotype

	Recombination fraction (θ)								
	0.00	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40
Family 1	2.98	2.56	2.24	1.91	1.59	1.27	0.95	0.65	0.37
Family 2	NI								
Family 3	0.90	0.81	0.72	0.62	0.52	0.41	0.30	0.19	0.09
Total	3.88	3.37	2.96	2.53	2.11	1.68	1.25	0.84	0.46

NI, not informative.

tion. Subsequent complications included pectus excavatum, scoliosis, high-grade myopia, lens dislocation and severe cardiovascular pathology necessitating mitral valve replacement and repair of ascending and descending aortic dissections. She died as consequence of congestive heart failure at age 17. Her parents and brother are clinically well, without signs of the Marfan syndrome except for isolated mitral valve prolapse in the brother, a finding seen in up to 8% of the general population⁸.

Nucleotide sequencing of PCR-amplified cDNA from patient E.S. revealed a G-to-C transversion at nucleotide 716 in one fibrillin allele (as numbered by C. Maslen *et al.*⁶). The corresponding amino-acid alteration is a substitution of proline (P) for arginine (R) at codon 239 (R239P) (Fig. 3). The mutation is not in either parent, as confirmed by allele-specific oligonucleotide (ASO) hybridization with PCR-amplified genomic DNA (Fig. 3), nor is it seen in the brother (data not shown). Paternity was confirmed using five highly polymorphic dinucleotide repeats⁹⁻¹². The mutant allele was not found by ASO analysis of PCR-amplified genomic DNA from 268 chromosomes from unrelated individuals without features of the Marfan syndrome.

ASOs specific for the normal and mutant alleles of patient E.S. were then used to screen PCR-amplified genomic DNA from 19 additional patients with sporadic disease, their parents, and an affected individual from 24 families with the Marfan syndrome. A second *de novo* occurrence of R239P was observed in a patient with sporadic disease. This mutation was confirmed by direct sequencing. The patient (B.C.) was diagnosed in infancy on the basis of arachnodactyly, joint hypermobility and ectopia lentis. Now aged 19 years, she has developed the additional manifestations of long-bone overgrowth, severe scoliosis, foot and leg deformities, mitral and tricuspid valve prolapse and regurgitation and severe dilation of the aortic root. Neither parent has any features of the Marfan syndrome. Paternity was confirmed as previously described for patient E.S..

The mutation in E.S. and B.C. is a nonconservative change, replacing a basic amino acid by the nonpolar α -imino acid, proline. Substitution of a proline residue may disrupt the secondary structure of the protein, including the proper formation of disulphide bonds, by introducing protein bending. In addition, the mutation occurs in a repetitive amino-acid sequence Gly-Xxx-Tyr/Phe-Xxx-Cys found at a periodicity of about every 40 amino acids throughout much of the characterized fibrillin-coding region⁶. The residue preceding cysteine, altered by mutation in patients E.S. and B.C., is often arginine (10/34), and never proline. This motif is part of a larger repetitive sequence in fibrillin that has homology to the tandem repeats in human epidermal growth factor (EGF) precursor⁶. A study of *Drosophila notch* gene mutations which result in single amino-acid substitutions in the *notch* 36 EGF-like repeats suggests that functional differences may exist among the tandemly repeated sequences¹³. These observations suggest that the mutation in E.S. and B.C. occurs in a functionally important region of the protein. Both patients with R239P have a severe phenotype with infantile presentation and overt manifestations of the ocular, skeletal and cardiovascular systems.

R239P occurs at a CpG dinucleotide but does not involve the C-to-T transition usually associated with such mutational 'hot-spots'. Although the predisposition (if any) for this recurrent

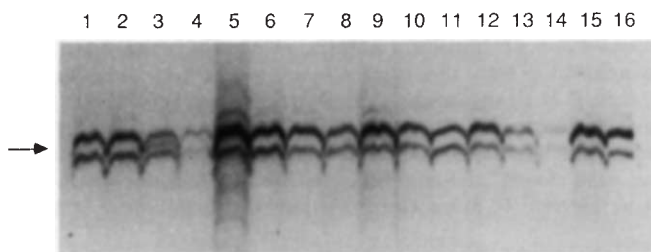


FIG. 2 SSCP analysis of PCR-amplified fibrillin cDNA from two clinically normal individuals (lanes 6 and 11) and 14 patients with isolated Marfan syndrome (lanes 1-5, 7-10, 12-16). The position of the anomalously migrating fragment in patient E.S. (lane 3) is shown by the arrow. The bands immediately above and below this position are seen in all individuals tested. Additional bands are occasionally observed in lanes with higher DNA concentrations. The prominent leading and trailing edges and irregular contour of each band are related to well configuration at the time of gel loading.

METHODS. Total RNA was extracted from cultured skin fibroblasts using the RNAzol B kit (Cinna/Biotech) according to instructions supplied by the manufacturer. Single-strand cDNA was synthesized according to standard protocol²¹ with slight modification (A. Hamosh *et al.*, manuscript in preparation). Fibrillin cDNA was amplified by PCR with primers F2-S 5'-GATATC-AATGAGTGAAGATG-3' and F2-AS 5'-GGCACACTGATACCTCCCT-3' (corresponding to nucleotides 388-408 and 849-831, respectively, as numbered by C. Maslen *et al.*⁶) using GeneAmp (Perkin-Elmer/Cetus) reagents. PCR conditions for this and all subsequently described reactions were: denaturing at 94 °C for 30 s, annealing at 56 °C for 30 s, then extension at 72 °C for 10 min. Samples were processed for SSCP analysis according to the methods of Orita *et al.*²² with modification, including direct incorporation of ³⁵S-labelled- α -dATP and ³⁵S-labelled- α -dCTP into the PCR product (L. C. Brody *et al.*, manuscript in preparation), loaded onto a 40 cm, 6% acrylamide, 10% glycerol, 1 $\times$ Tris-borate-EDTA gel, and electrophoresed at room temperature, 40 W for 7 h.

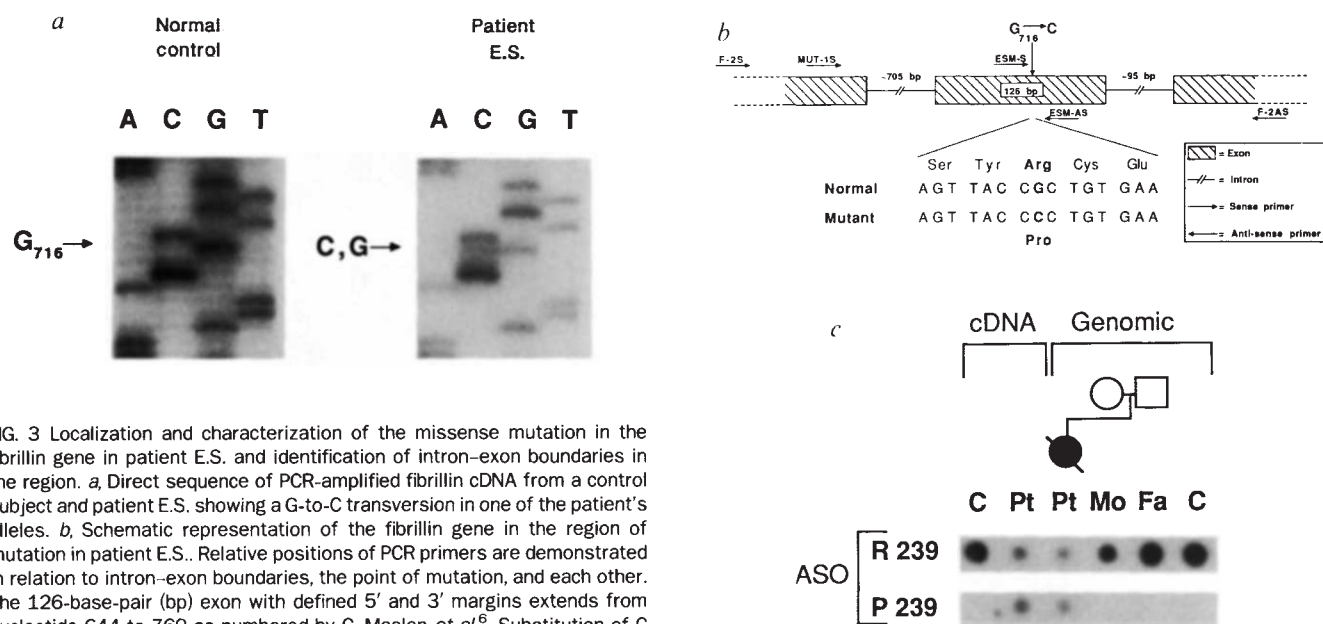


FIG. 3 Localization and characterization of the missense mutation in the fibrillin gene in patient E.S. and identification of intron-exon boundaries in the region. **a**, Direct sequence of PCR-amplified fibrillin cDNA from a control subject and patient E.S. showing a G-to-C transversion in one of the patient's alleles. **b**, Schematic representation of the fibrillin gene in the region of mutation in patient E.S.. Relative positions of PCR primers are demonstrated in relation to intron-exon boundaries, the point of mutation, and each other. The 126-base-pair (bp) exon with defined 5' and 3' margins extends from nucleotide 644 to 769 as numbered by C. Maslen *et al.*⁶. Substitution of C for G occurs in one allele of patient E.S. at nucleotide 716. Exon sequence and the corresponding amino-acid sequence of the normal and mutant alleles in the region of the mutation are shown at the bottom of the figure demonstrating an arginine to proline substitution at codon 239. **c**, Dot blot of PCR-amplified cDNA and genomic DNA from control subjects (C), patient E.S. (Pt), her mother (Mo) and father (Fa) hybridized to ³²P-labelled ASOs. Normal sequence ASO R239 (5'-TTCACAGCGGTAACCTT-3') and mutant sequence ASO P239 (5'-AAGTTACCCCTGTGAA-3'), corresponding to nucleotides 723-708⁶ and 708-723⁶, respectively, were designed to create C-C mismatches when hybridized to patient DNA thus promoting maximal instability of mismatch heteroduplexes.

METHODS. **a**, **b**, Patient and control subject cDNA was amplified using PCR primers F-2S and F-2AS (described in legend to Fig. 2) and 7-deaza-dGTP (Boehringer Mannheim). Sequencing with primers MUT-1S (5'-GACGAA-GGCTATGAAAGTG-3'; nucleotides 598-616⁶) and F-2AS was done using an

automated DNA sequencer (Applied Biosystems 373A) and manually using the Sequenase kit (USB) with dITP. Intron-exon boundaries were localized by PCR amplification of genomic DNA from clinically normal individuals using primers MUT-1S and F-2AS which yielded a larger DNA fragment than predicted from the cDNA sequence (1050 bp versus 251 bp). Sequencing, using primers ESM-S (5'-TTTGCCATAACACAGAGGG-3'; nucleotides 689-707⁶) and ESM-AS (5'-GATGGCCAGGCGGGCA-3'; nucleotides 739-724⁶) allowed identification of boundaries and exon sizing. **c**, Preparation of dot blots was done as previously described²³. Filters were hybridized in 5 × SSPE (0.9 M NaCl, 5 mM NaH₂PO₄, 5 mM sodium-EDTA pH 7.4), 0.5 % SDS, 0.1 % BSA, 0.1 % polyvinyl pyrrolidone and 0.1 % Ficoll at 46 °C for 60 min with 5 × 10⁶ c.p.m. ml⁻¹ ³²P-end labelled ASO. Filters were then washed twice at 47 °C for 10 min in 5 × SSPE, 0.1 % SDS.

alteration remains to be elucidated, the finding of a recurrent *de novo* mutation that is not a transition at a CpG dinucleotide is not a novel phenomenon, as illustrated by recent experience with the factor VIII gene^{14,15}.

There are several explanations for the failure to identify gross alterations on Southern analysis. Point mutations, or small rearrangements, may make up most of the defects in the fibrillin gene. Alternatively, gross rearrangements which interrupt fibrillin synthesis from one allele may have fewer or different clinical manifestations than mutations resulting in production of an abnormal protein, as has been observed in type I collagen¹⁶. Finally, as specific gene sequences may be prone to rearrangement^{17,18}, gross alterations may yet be found in the 30% of fibrillin-coding sequence still to be cloned. These issues may be resolved with the identification and characterization of addi-

tional mutations in the gene, expression studies and subsequent analysis of phenotype-genotype correlations.

In multiplex families in which linkage to the fibrillin locus and flanking markers can be established, prenatal or presymptomatic diagnosis may now be offered by linkage methods. Moreover, it is possible that direct mutational analysis will become feasible as more is learned about the nature of fibrillin mutations in the Marfan syndrome. Similar approaches may be used to assess the role of fibrillin mutations in inter- and intrafamilial variability in this disorder, in phenotypically similar syndromes, and in families with isolated features of this disease such as mitral valve prolapse, medial degeneration of the aorta and scoliosis. The result of such efforts will be more precise nosology, diagnosis and interventions for these and other related disorders of connective tissue. □

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Homozygous prion protein genotype predisposes to sporadic Creutzfeldt–Jakob disease

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THE human prion diseases, Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler syndrome (GSS), are neurodegenerative diseases that are unique in being both infectious and genetic. Transmission of both diseases and the animal spongiform encephalopathies (for example, scrapie and bovine spongiform encephalopathy) to experimental animals by intracerebral inoculation with brain homogenates is well documented¹. Despite their experimental transmissibility, missense and insertional mutations in the prion protein gene are associated with both GSS and familial CJD, demonstrating that the human familial cases are autosomal dominant diseases^{2–6}. More than 80% of CJD cases occur sporadically, however, and are not known to be associated with mutations. Here we report that 21 of 22 sporadic CJD cases and a further 19 of 23 suspected sporadic CJD cases are homozygous at the polymorphic amino-acid residue 129; 51% of the normal population are heterozygous at this site. We argue that homozygosity predisposes towards sporadic CJD and that this directly supports the hypothesis that interaction between prion protein molecules underlies the disease process.

The prion protein (PrP) gene encodes a normal cell-surface glycoprotein found on neuronal cells, lymphocytes and other tissues^{7,8}. Coding mutations (Fig. 1) have been found in familial CJD, GSS and also in atypical familial dementias now known to be part of the spectrum of inherited prion disease^{9,10}. These mutations occur only in affected or at-risk members of these families and have not been found in the normal population. Furthermore, transgenic mice encoding the equivalent mutation to that reported in GSS spontaneously develop spongiform encephalopathy¹¹. But most cases of CJD are sporadic and their aetiology has still to be determined; environmental prion infection remains a possible explanation for these cases. A few cases of CJD have occurred as a result of exposure to infective

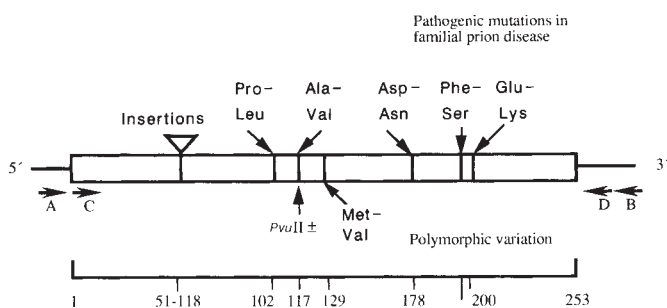


FIG. 1 Diagram of the PrP gene open reading frame showing the position of known mutations and polymorphisms. Amino acids are designated in the three-letter code; Pro–Leu indicates a mutation from proline to leucine at that site. The positions of four oligonucleotide primers used for PCR amplification (A, B, C and D) are marked by arrows. Numbers indicate codon positions.

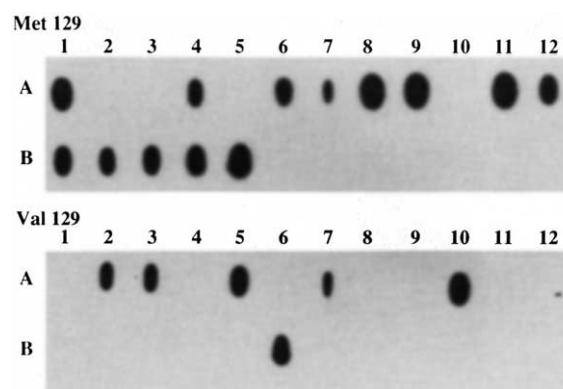


FIG. 2 Autoradiograms demonstrating codon-129 genotypes of 18 samples by allele-specific oligonucleotide hybridization. 'A' and 'B' are row designations on the slot blot manifold; slot blot position B7 is a control without DNA. **METHODS.** Peripheral blood lymphocytes or fresh brain tissue was obtained on 22 sporadic CJD cases. In addition, a further 23 cases of clinically suspected CJD (referred to the UK CJD surveillance study) were analysed. Genomic DNA was extracted using standard techniques. The PrP gene open reading frame was amplified by PCR. The PCR mixture consisted of 1 µg genomic DNA, 25 pmol of each oligonucleotide primer, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM MgCl₂, 0.01% gelatin, 200 µM of each deoxynucleoside triphosphate, 5% dimethylsulphoxide and 2.5 units Taq polymerase (Amplitaq, Perkin Elmer–Cetus) in a total volume of 100 µl; reaction samples were overlaid with 100 µl mineral oil. PCR cycling conditions were 94 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min for 35 cycles in a Techne PHC-2 thermal cycler. Synthetic primers (Fig. 1) for amplification were, A: 5'-ACTGAGAATTCTGACATTCTCCTCTTCA-3'; B: 5'-TACTGAGGATCCCTC-AAGCTGGAAAAAGA-3'; C: 5'-GTGGCCACATGGAGTGACCTGGGCTC-3'; D: 5'-GAAAGATGGTGAAACAGGAAGACC-3'. The PCR product (10 µl) was denatured with 40 µl 0.3 M NaOH, 25 mM EDTA for 5 min, then neutralized with 50 µl 0.6 M Tris–HCl (pH 7.4), 1.5 M NaCl before vacuum blotting onto nylon filters (Hybond-N, Amersham). After baking, filters were prehybridized in 6 × SSPE (SSPE is 0.18 M NaCl, 0.01 M sodium phosphate, pH 7.7, 0.001 M EDTA), 0.1% BLOTTO (5% not fat dried milk, 0.02% sodium azide), 0.1% SDS, 1 mM EDTA and 100 µg ml⁻¹ denatured salmon sperm DNA at 36 °C for 3 h. Filters were hybridized for 16 h under the same conditions in the presence of 10⁶ c.p.m. per ml ³²P-end-labelled oligonucleotide. Filters were washed in 3 M tetramethylammonium chloride, 50 mM Tris–HCl (pH 8.0), 2 mM EDTA and 0.1% SDS at 36 °C for 15 min, followed by two further washes at 47 °C for 15 min before autoradiography for 4 h. Allele-specific oligonucleotides were Met-129: 5'-CGGCTACATGCTGGG-3', Val-129: 5'-CGGCTACGTGCTGGG-3'.

material. These iatrogenic cases have arisen as a result of the use of contaminated intracerebral electrodes, dura mater and corneal grafts, and treatment with human cadaveric derived pituitary hormone¹².

As well as the pathogenic mutations, there is a polymorphism at codon 129 that results in the substitution of valine for methionine¹³. We have previously reported that the normal population has a genotype frequency of 37% Met 129/Met 129, 51% heterozygous and 12% Val 129/Val 129 (sample size, 106 individuals)¹⁴. We have examined all cases in the United Kingdom who developed CJD following treatment with human cadaveric pituitary hormone and reported that there was a significant excess of Val-129 homozygotes¹⁴. This suggested that in populations exposed to contaminated hormone preparations, those with a Val-129 homozygous genotype may be at increased risk of developing CJD. A prediction from this result is that if sporadic CJD were due to environmentally acquired contamination, then there would also be a significant increase in Val-129 homozygosity. We tested the genotype of 22 sporadic CJD cases and a further 23 suspected sporadic CJD cases (from the United Kingdom CJD surveillance study). All cases were from the same caucasian population as the controls. Samples were analysed by oligonucleotide-specific hybridization (Fig. 2) to PrP open reading frame amplified using the polymerase chain reaction

(PCR). Of the 22 CJD cases, 16 were homozygous for Met 129, 5 homozygous for Val 129 and only one was a heterozygote, while of the suspected CJD cases, 11 were Met-129 homozygotes, 4 were heterozygotes and 6 were Val-129 homozygotes. The two groups had homozygosity frequencies of 95.5% and 82% respectively, and differ significantly from the normal population frequency of 49% homozygotes, although the increase in valine homozygotes (22%) originally tested for is not significant. For the CJD group, 95.5% homozygosity represents a $\chi^2 = 14.17$ ($P = 0.00017$), and for the suspected CJD group, $\chi^2 = 7.29$ ($P = 0.0069$). When combined, these figures give a $\chi^2 = 19.4$ ($P = 0.00001$). (χ^2 was calculated using Yates correction.) Interestingly, the single heterozygous case in the CJD group may in fact be a familial case as subsequent examination of the case records reveal that this patient's father died with dementia.

The analysis was repeated with a second, nonoverlapping PCR primer pair to exclude the possibility that a mutation affecting hybridization of one primer could have accounted for the results. Two cases homozygous at codon 129 were heterozygous for an uncommon *PvuII* noncoding polymorphism at codon 117 (ref. 15), however, directly demonstrating that both alleles had been amplified by our method. This was shown by oligonucleotide hybridization and confirmed by digestion with restriction enzymes (Fig. 3). Codon 129 genotypes were confirmed using the restriction endonucleases *NspI* and *MaeII* to exclude the possibility that mutations near codon 129 interfering with oligonucleotide hybridization to one allele could account for the results (Fig. 4).

The importance of the amino acid at position 129 is further demonstrated by the finding of a correlation between the codon 129 genotype and age of death in an extended pedigree with inherited prion disease¹⁶. The affected individuals all possess a 144-base-pair insert in the open reading frame lying on a Met-129 allele. Individuals homozygous for Met 129 (though heterozygous for the insertion) died at a significantly earlier age ($P < 0.01$) than those who were heterozygous at codon 129.

Several lines of evidence suggest that an abnormal, partly protease-resistant, isoform of the prion protein, PrP^{Sc}, is an essential and possibly the sole constituent of the infective agent¹⁷. The molecular difference between PrP^{Sc} and the normal cellular isoform (PrP^C) remains to be determined, although the change is assumed to be post-translational as no differences in primary structure have been identified. Our data support the idea that the disease process involves the formation of PrP^C to PrP^{Sc} remains obscure. One hypothesis is that PrP^{Sc} can, by formation of a dimer with PrP^C, catalyse the conversion of PrP^C to PrP^{Sc} to produce a chain reaction of conformational transfor-

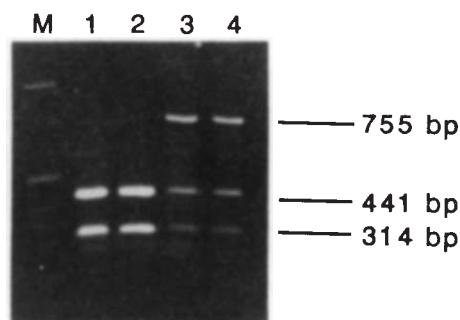


FIG. 3 Agarose gel (1.5%) electrophoresis of PCR-amplified PrP open reading frame (using primers C and D) digested with *PvuII* under standard conditions. The PCR product is 755 base pairs (bp) long. *PvuII* digestion products are 441 bp and 314 bp. Samples in lanes 1 and 2 are homozygous for the *PvuII* restriction site; both alleles are digested. Lanes 3 and 4 shows the two samples heterozygous for the *PvuII* restriction site. Lane M, 1-kb DNA size-calibration ladder (BRL).

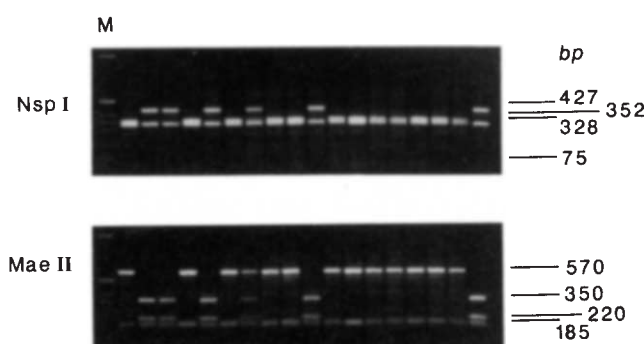


FIG. 4 Codon 129 genotypes of the same 18 samples used for Fig. 2 assessed by *NspI* and *MaeII* digestion. *NspI* cuts codon 129 when it encodes Met. Together with an *NspI* site at codon 155, the 755-bp product of a Met allele is digested to give three bands of 352, 328 and 75 bp on agarose gels (1.5%). Alleles encoding Val at codon 129 retain the 328-bp band plus a band of 427 bp. *MaeII* cuts codon 129 when it encodes Val. Together with a *MaeII* site at codon 203, the 755-bp PCR product of a Val allele is digested to give three bands of 350, 220 and 185 bp. Met alleles are digested to give the 185-bp band plus one of 570 bp. Lane M, 1-kb DNA size-calibration ladder (BRL).

mation which leads progressively towards the disease state^{18,19}. During experimental transmission or iatrogenic inoculation, the PrP^{Sc} form is introduced from exogenous sources. In the familial cases, prion protein containing the known pathogenic mutations may spontaneously produce PrP^{Sc}, although the spontaneous conversion is presumably still a rare event. If the development and progression of the disease depend on the association of protein as dimers, sequence identity may be important for the efficiency of the conversion. Heterozygotes may therefore be partially protected against the disease because of the increased formation of heterodimers, whereas homozygotes would be more susceptible. The partial barrier to interspecies transmission²⁰ that is seen experimentally may be an extreme form of such an effect, in that heterodimer formation that is even less favoured is required to trigger an infection.

The genotype associated with genetic predisposition to CJD on exposure to environmental prions, Val 129/Val 129, was not present to the same degree of excess in our sporadic CJD population as was found in the iatrogenic cases, suggesting that most sporadic cases are not acquired from the environment. Alternative explanations for the aetiology of sporadic CJD include germ-like mutations in a minority, somatic mutations resulting in formation of PrP^{Sc} in the cells carrying the mutation, and spontaneous conversion of PrP^C to PrP^{Sc} as a rare stochastic event. These explanations are more consistent with the observed uniform worldwide incidence of sporadic CJD irrespective of local scrapie prevalence²¹.

That 40 out of 45 individuals were homozygous at residue 129 is consistent with the above model. The generation of PrP^{Sc} in a cell by whatever method would be more likely to result in disease if this occurred in a homozygous individual (whether they carried Met or Val at position 129). The results from the sporadic, iatrogenic and autosomal dominant cases together provide support for the involvement of protein-protein interactions in the disease process and imply that amino acid 129 is at an important position in PrP for dimer formation. □

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Recognition and plasma clearance of endotoxin by scavenger receptors

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LIPID A is the active moiety of lipopolysaccharide (LPS, also referred to as endotoxin), a surface component of Gram-negative bacteria that stimulates macrophage activation and causes endotoxic shock^{1,2}. Macrophages can bind, internalize and partially degrade LPS, lipid A and its bioactive precursor, lipid IV_A (refs 3–7). We report here that lipid IV_A binding and subsequent metabolism to a less active form by macrophage-like RAW 264.7 cells is mediated by the macrophage scavenger receptor. Scavenger-receptor ligands inhibit lipid IV_A binding to, and metabolism by, RAW cells, and lipid IV_A binds to type I and type II bovine scavenger receptors on transfected Chinese hamster ovary cells. Although *in vitro* competition studies with RAW cells indicate that scavenger receptor binding is not involved in LPS or lipid IV_A-induced stimulation of macrophages, *in vivo* studies show that scavenger-receptor ligands greatly inhibit hepatic uptake of lipid IV_A in mice. Thus, scavenger receptors expressed on macrophages may have an important role in the clearance and detoxification of endotoxin in animals.

To identify macrophage receptors for lipid IV_A (Fig. 1) and related lipids, 4' [³²P]lipid IV_A was prepared, and assays for binding and metabolism were performed as previously described^{5–7}. The high-affinity binding (EC_{50} for saturation ~25–50 nM; ref. 6) of 4' [³²P]lipid IV_A to RAW cells is very similar to the macrophage binding of scavenger receptor ligands⁸. Acetylated low-density lipoprotein (AcLDL, Fig. 2a), as well as maleylated BSA (M-BSA), polyinosinic acid (poly (I)), polyinosinic: polycytidylic acid, fucoidan and dextran sulphate (not shown), effectively block specific binding and uptake of lipid IV_A at 22 °C (and 0 °C, not shown), whereas LDL (Fig. 2a), as well as heparin, polyglutamate, polyphosphate glass or polycytidylic acid (poly (C)) (not shown) do not. Other similarities include divalent cation independence⁸ and inducibility in THP-1 cells by phorbol esters^{9,10}. Both lipid IV_A and ReLPS (ref. 1) inhibit uptake of [¹²⁵I]-AcLDL by RAW cells under the conditions used in Fig. 2a (data not shown).

At 37 °C, RAW cell-associated lipid IV_A is dephosphorylated at position 1 in a time- and temperature-dependent fashion, generating 4' [³²P]-monophosphoryl-lipid IV_A (M in Fig. 2b). This may be physiologically relevant, because loss of this phosphate greatly lowers bioactivity¹¹. Dephosphorylation is inhibited by the scavenger-receptor ligands AcLDL, poly (I) and M-BSA, but not by LDL, poly (C) or BSA (Fig. 2b). Dephosphorylation was also blocked by 10 µg ml⁻¹ chloroquine (Fig. 2b), as is scavenger receptor-mediated degradation of lipoproteins⁸.

To determine whether lipid IV_A binds directly to scavenger receptors, we examined its binding and metabolism by Chinese hamster ovary (CHO) cells stably transfected with complementary DNAs encoding either the type I (CHO[bSR-I]) or type II (CHO[bSR-II]) bovine scavenger receptors^{12–14}. The transfected CHO cells have much greater AcLDL-inhibitable uptake (Fig. 2c) and metabolism (Fig. 2d) of 4' [³²P]lipid IV_A than the untransfected controls, and the ligand specificity of lipid IV_A metabolism in either type I (not shown) or type II (Fig. 2e) transfectants is identical to that in RAW cells (Fig. 2b). The small amount of activity in untransfected cells is probably due to low levels of endogenous scavenger-receptor activity¹⁸. Because the CHO[bSR-II] cells express about twice the number of receptors than the CHO[bSR-I] cells¹⁴, it seems that there is no great difference between the abilities of the two receptors to bind lipid IV_A. RAW cells metabolize a much larger fraction of cell-associated lipid IV_A than the transfectants in uptake experiments performed at nanomolar ligand concentrations (see

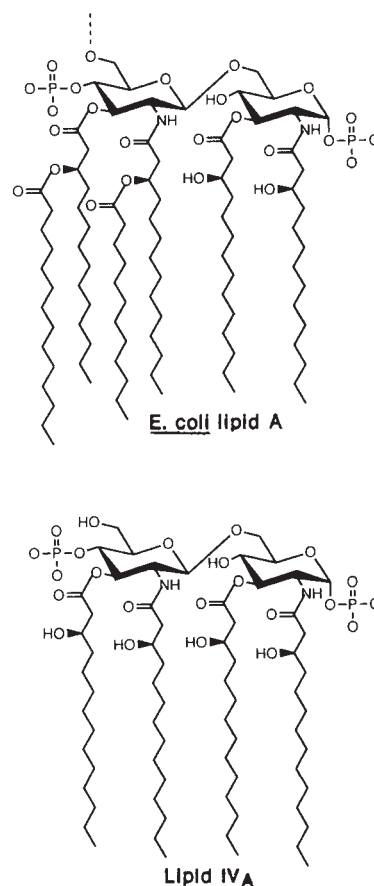


FIG. 1 Lipid A moiety of LPS and precursor lipid IV_A. The 6' position of lipid A (dotted line) is the site of attachment of the core oligosaccharide region of LPS¹. Lipid IV_A (bottom) is a key biosynthetic precursor of lipid A¹. The 4'-phosphate, located on the nonreducing sugar (left), can be radiolabelled with a specific kinase¹⁶. The 1-phosphate (right) can be removed by animal cells after internalization to yield 4'-monophosphoryl lipid IV_A.

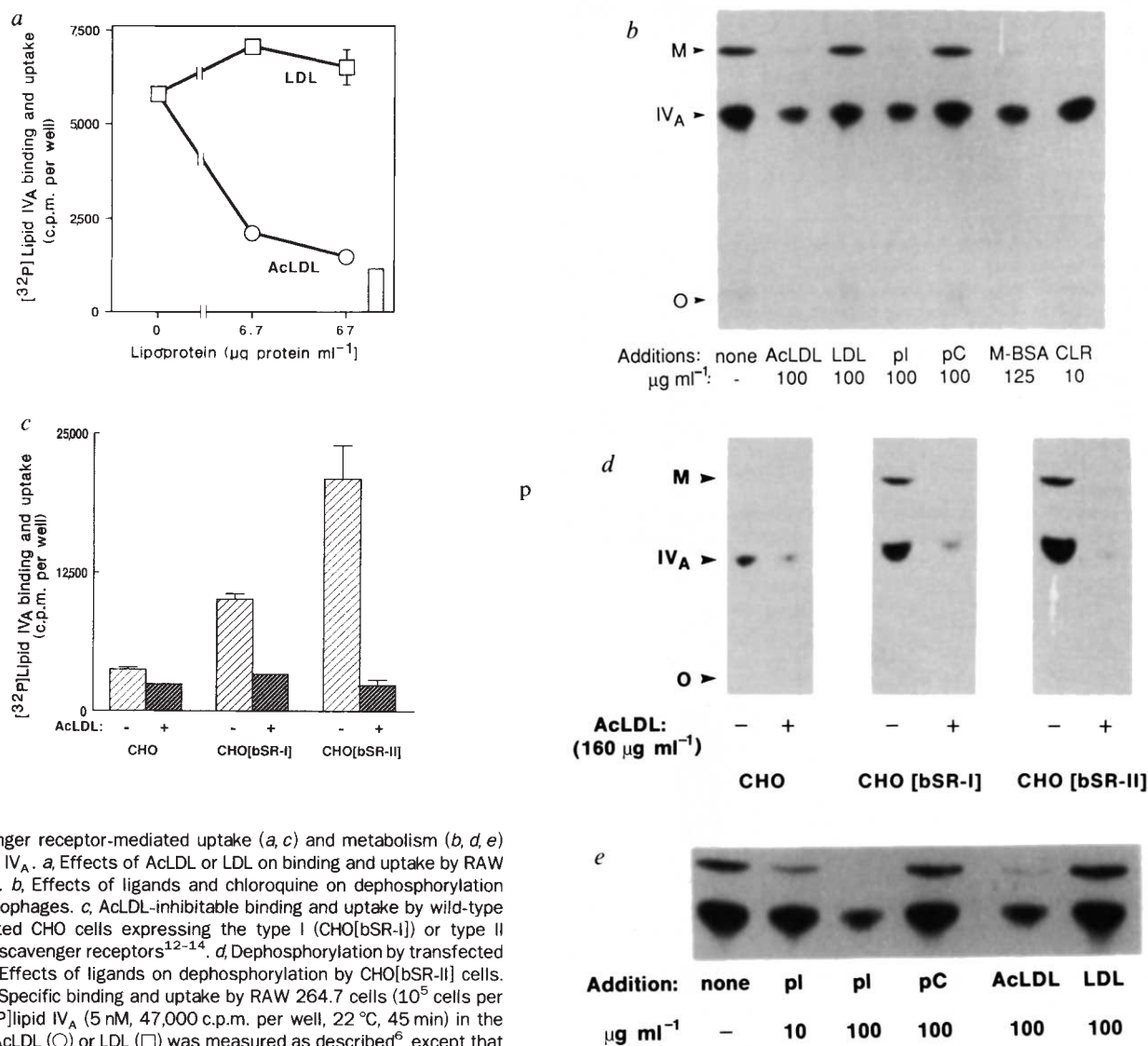


FIG. 2 Scavenger receptor-mediated uptake (a, c) and metabolism (b, d, e) of 4'[^32P]lipid IV_A. a, Effects of AcLDL or LDL on binding and uptake by RAW macrophages. b, Effects of ligands and chloroquine on dephosphorylation by RAW macrophages. c, AcLDL-inhibitable binding and uptake by wild-type and transfected CHO cells expressing the type I (CHO[bSR-I]) or type II (CHO[bSR-II]) scavenger receptors¹²⁻¹⁴. d, Dephosphorylation by transfected CHO cells. e, Effects of ligands on dephosphorylation by CHO[bSR-II] cells. METHODS. a, Specific binding and uptake by RAW 264.7 cells (10⁵ cells per well) of 4'[^32P]lipid IV_A (5 nM, 47,000 c.p.m. per well, 22 °C, 45 min) in the presence of AcLDL (○) or LDL (□) was measured as described⁶, except that the assay buffer (Ham's F12 medium with 0.5% BSA) contained 10 mM HEPES, pH 7.4. The open bar indicates nonspecific binding and uptake in the presence of 10 μg ml⁻¹ unlabelled lipid IV_A. b, RAW monolayers (10⁵ cells per well) in 12-well tissue culture plates (Costar) were incubated as in a with 4'[^32P]lipid IV_A (5 nM, 250,000 c.p.m. per well) at 37 °C for 3 h in the presence of the indicated ligands, or chloroquine (CLR), which were prepared as previously described¹⁵⁻¹⁷ or purchased from Sigma. Monolayers were washed with assay buffer, and extracted with 100 μl of 2% Triton X-100/5 mM EDTA. Chromatograms of extracts (10 μl on silica gel 60 (E. Merck) developed in chloroform/pyridine/88% formic acid/H₂O (40:60:16:5, v/v)) were visualized by autoradiography. The origin, 4'[^32P]lipid IV_A, and 4'-monophosphoryl-[^32P]lipid IV_A are labelled 'O', 'IV_A' and 'M'. With

no additions, about 40% of the monolayer-associated lipid IV_A was dephosphorylated. c, d, Duplicate monolayers of wild-type CHO and transfected CHO[bSR-I] and CHO[bSR-II] cells¹²⁻¹⁴ (2 × 10⁵ per well, 24 well plates) were incubated with 4'[^32P]lipid IV_A (1 μM, 220,000 c.p.m. per well) at 37 °C for 3 h with or without AcLDL (160 μg protein ml⁻¹) and cell-associated ³²P was extracted as in (b) and either counted directly (c) or samples (10 μl) were subjected to TLC and autoradiography (d). e, Monolayers of CHO[bSR-II] cells were incubated with 4'[^32P]lipid IV_A (1 μM, 220,000 c.p.m. per well) and the indicated additions and processed as in b. In a and c, values and error bars represent the mean and range of duplicate determinations.

Fig. 2b). This altered efficiency could be due to species (mouse versus hamster or bovine) or cell-type (CHO versus RAW macrophage) differences.

Taken together, these data show that scavenger receptors mediate the binding and metabolism of lipid IV_A. Lipid IV_A is one of a large group of anionic lipids, including LPS and acidic phospholipids such as phosphatidylserine (data not shown and ref. 19) which are scavenger-receptor ligands.

We next examined the physiological relevance of scavenger receptors to LPS signalling in macrophages^{1,2,20} and to LPS clearance *in vivo* by the liver. Scavenger receptor-inhibiting concentrations of AcLDL (352 μg protein ml⁻¹, Fig. 3 inset) could not induce TNF-α production (Fig. 3), trigger mobilization of arachidonic acid (not shown) or alter lipid IV_A or ReLPS stimulation of TNF-α release (Fig. 3, and data not

shown). Thus, scavenger receptors are probably not centrally involved in the LPS-induced activation of RAW or other macrophage cells.

LPS in the bloodstream normally arises from the gut or from sites of infection². The liver rapidly clears circulating LPS²², and both Kupfer and sinusoidal endothelial cells in the liver have very high scavenger-receptor activity^{8,10,23-26}. We measured the rapid and extensive *in vivo* hepatic uptake of [^32P]lipid IV_A in mice in the absence or presence of competing scavenger-receptor ligands (Table 1). As much as 79% of the total injected [^32P]lipid IV_A is found in the liver in as little as 10 min after injection (experiment 2). The amount of injected lipid present in the liver due to trapped blood would be expected to account for less than ~10% of the observed uptake²⁶. Coinjection of the ligands AcLDL, poly (I) or M-BSA, but not the corresponding

TABLE 1 Specific inhibition of liver uptake of 4-[³²P]lipid IV_A by scavenger receptor ligands in mice

Addition: (amount):	None* (—)	AcLDL (100 or 330 µg)†	Coinjected scavenger receptor ligands or controls			M-BSA (1,500 µg)	BSA
			LDL (330 µg)	pl (330 µg)	pC (330 µg)		
			c.p.m. per 100 mg liver (% of vehicle control)				
Experiment 1	20,301 (100)	12,555 (61.8)	22,205 (109)	—	—	—	—
Experiment 2	47,784 (100)	33,734‡ (70.6)	44,609 (93.4)	29,293‡ (61.3)	41,948 (87.8)	—	—
Experiment 3	23,262 (100)	—	—	15,494‡ (66.6)	23,495 (101)	10,890‡ (46.8)	23,735 (102)

In three separate experiments, ether anaesthetized, female BALB/c mice (20–25 g) were injected in the retroorbital plexus with 200 µl phosphate buffered saline (PBS) containing 0.5% (w/v) BSA and [³²P]lipid IV_A (made from a sonicated stock solution of labelled IV_A (refs 6, 7)) added to the BSA solution and then prefiltered through a 0.22 µm µ-star filter (Costar, Cambridge, Massachusetts, immediately before injection) and the indicated additions of scavenger receptor ligands or corresponding negative controls or buffered saline vehicle alone (none). Ten (experiments 2 and 3) or 15 (experiment 1) min after injection, mice were reanaesthetized and killed by cervical dislocation. Liver specimens were immediately dissolved with Protosol (NEN, Boston) at 60 °C for ~20 h, bleached with 30% H₂O₂ and the radioactivity determined by scintillation counting. The injected doses of [³²P]lipid IV_A were 13 ng (400,000 c.p.m.), 14 ng (600,000 c.p.m.) and 8 ng (350,000 c.p.m.) for experiments 1–3, respectively. Using a blood volume of roughly 1.5 ml per mouse²⁷, we estimate that the initial blood concentrations of lipid IV_A were around 3.5–7 nM. The values shown are the means of duplicate (experiment 1 and no-addition control ('none') in experiment 3) or triplicate (all other) mice. Ranges (duplicates) or s.d. (triplicate) for all conditions fell between 2–12% of the respective means. pl, poly (I); pC, poly (C).

* Total liver uptake of lipid IV_A as fraction of injected amount, based on a rough liver weight of 1 g per mouse: experiment 1, 51%; experiment 2, 79%; experiment 3, 66%.

† 100 µg per mouse in experiment 1, 330 used in experiment 2.

‡ $P < 0.005$ versus corresponding control addition and $P < 0.05$ versus no-addition ('none') control; student's *t*-test.

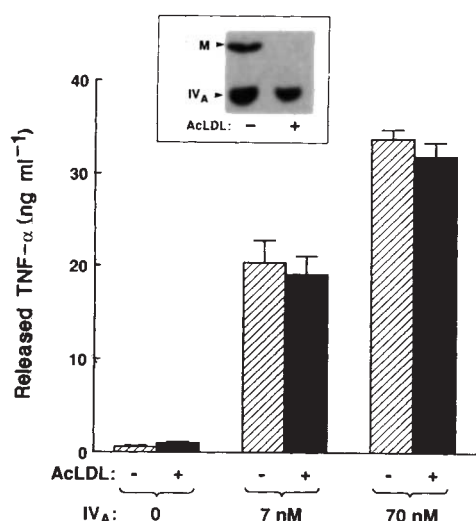


FIG. 3 Effects of AcLDL on TNF-α release and lipid IV_A-stimulated TNF-α release by RAW 264.7 cells.

METHODS. RAW 264.7 monolayers (2.5×10^5 cells per well) in 48-well tissue culture plates were washed three times with 0.5 ml Ham's F12 medium containing glutamine, bicarbonate buffer (Whittaker Bioproducts) and 0.5% human serum albumin (Worthington Biochemical, Freehold, New Jersey), overlaid with 0.25 ml of the same buffer containing the indicated concentrations of lipid IV_A with (+) or without (–) 325 µg protein per ml AcLDL, and allowed to incubate for 3 h at 37 °C in a 5% CO₂ incubator. Secreted TNF-α was measured by capture radioimmunoassay using TNF-α monoclonal antibody TN19.12 (ref. 21). Insert: In parallel, washed monolayers were overlaid with 0.25 ml of buffer containing 70 nM 4-[³²P]lipid IV_A with (+) or without (–) 352 µg protein per ml AcLDL and dephosphorylation was assayed by TLC and autoradiography as described in Fig. 2b.

negative controls, significantly and reproducibly reduce hepatic uptake of [³²P]lipid IV_A (Table 1). The ligand-dependent decrease in [³²P]lipid IV_A uptake is 30–50% of the total uptake, suggesting either that there may be additional scavenger receptor-independent uptake mechanisms or that the amounts of competing ligands we used were not sufficient to block all the very high hepatic scavenger-receptor activity. This quantitatively significant scavenger receptor-mediated [³²P]lipid IV_A clearance occurs at lipid IV_A concentrations (3.5–7 nM; see Table 1) similar to those required for the interaction of endotoxic lipids

with lipopolysaccharide-binding protein, LBP (ref. 28). LBP, a serum protein, seems to be involved in the recognition of endotoxin by some eukaryotic cells, and in their subsequent activation^{29,30}.

These results suggest that scavenger receptors may be important in the clearance and degradation of endotoxic lipids, possibly to protect against endotoxic shock. Because of the distinctive, broad ligand-specificity of scavenger receptors, they may help to defend against a wide variety of pathogenic agents by speeding their removal from the circulation. □

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Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6

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WILD-TYPE p53 protein has many properties consistent with its being the product of a tumour suppressor gene¹⁻³. Although the normal roles of tumour suppressor genes are still largely unknown, it seems that they could be involved in promoting cell differentiation⁴⁻⁶ as well as in mediating growth arrest by growth-inhibitory cytokines⁷⁻⁹. Hence, the abrogation of wild-type p53 expression, which is a common feature of many tumours, could eliminate these activities. We have now tested this notion by restoring the expression of p53 in a murine myeloid leukaemic cell line that normally lacks p53. The use of a temperature-sensitive p53 mutant¹⁰ allowed us to analyse cells in which the introduced p53 had either wild-type or mutant properties. Although there seemed to be no effect on differentiation, the introduction of wild-type p53 resulted in rapid loss of cell viability in a way characteristic of apoptosis (programmed cell death). The effect of wild-type p53 was counteracted by interleukin-6. Thus products of tumour suppressor genes could be involved in restricting precursor cell populations by mediating apoptosis.

Cells of clone S6 (ref. 11) of the murine myeloid leukaemic line M1 (refs 12-14) express neither p53 protein (Fig. 1) nor p53 messenger RNA (data not shown). DNA encoding a temperature-sensitive mutant of murine p53 (Ala to Val change at position 135)¹⁰ was stably introduced into M1 cells by electroporation¹¹, along with plasmid pSV2neo, conferring resistance to G418 (ref. 15). Drug-resistant clones expressing p53 (Fig. 1) were further analysed.

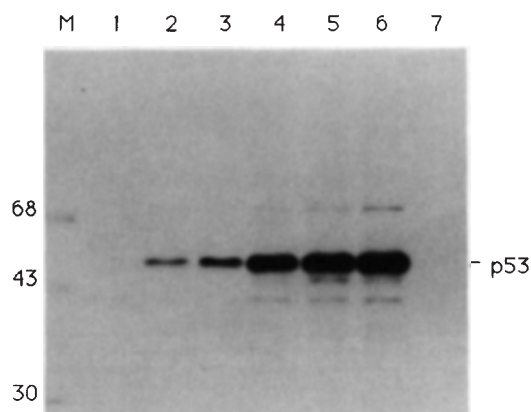


FIG. 1 Detection of p53 in transfected M1 cells. Cells of the parental M1 line, clone S6 (lane 1) and of clones transfected with plasmids encoding the Val135 p53 mutant under either the simian virus 40 (SV40) early control region (pSVp53cG3)⁴¹ or the Harvey sarcoma virus long terminal repeat (LTR) (pLTRcGval135)¹⁰ (lanes 2-7, see text), were labelled for 3 h with [³⁵S] methionine and their proteins analysed as described before¹⁰. In each case, a cell extract aliquot containing 5×10^6 acid-insoluble c.p.m. was reacted with the p53-specific monoclonal antibody PAb421, except in lane 7, where control hybridoma culture medium was used. Lines represented: lane 2, SV-1; 3, SV-4; 4, LTR-1; 5, LTR-13; 6 and 7, LTR-14. M, protein size markers (relative molecular masses ($\times 10^{-3}$) are indicated).

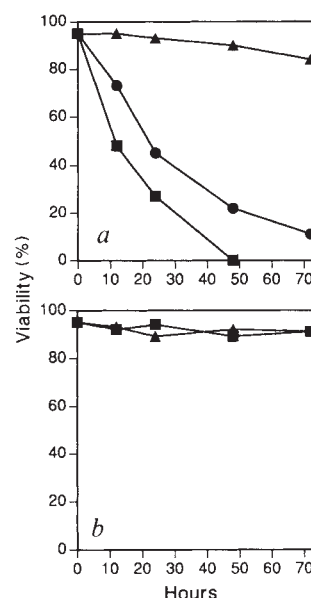


FIG. 2 Induction of cell death by the wild-type activity of the Val135 mutant. Parental M1 cells ($\blacktriangle$), as well as clones derived by transfection of M1 with the Val 135 mutant were incubated at either 32.5 °C (a; at time 0 the cells were transferred from 37.5 °C–32.5 °C) or 37.5 °C (b), and viability was determined at each time point by trypan-blue exclusion. $\bullet$, clone SV-1; $\blacksquare$, clone LTR-1 (see Fig. 1).

The Val 135 mutant behaves like other p53 mutants at 37.5 °C, but like wild-type p53 at 32.5 °C (refs 10, 16, 17). The behaviour of transfected M1 cells was therefore compared at both temperatures. In standard culture medium (RPMI-1640, 10% fetal calf serum) no spontaneous differentiation was observed at either temperature (data not shown). Thus, the activation of wild-type p53 at 32.5 °C did not promote differentiation. The transfectants, however, rapidly lost viability at 32.5 °C (Fig. 2a) but not at 37.5 °C (Fig. 2b). Similar results were obtained with additional independently derived clones expressing the Val 135 mutant, whereas no death was seen at 32.5 °C with parental M1 cells (Fig. 2a) or with cells transfected either with pSV2neo alone or with plasmid pLTRcGp53, encoding a non-temperature-sensitive p53 mutant with Phe at position 132 (ref. 10; see Fig. 4a).

Cells may die by necrosis (as a result of injury, infection or some other external stress), or through the more specific process of apoptosis (programmed cell death)¹⁸. Apoptosis is probably involved in restricting the sizes of immature progenitor cell pools in the absence of relevant growth-promoting stimuli¹⁹⁻²¹. Apoptotic cells have a distinct morphology, and show a characteristic pattern of DNA fragmentation resulting from cleavage of nuclear DNA in internucleosomal regions¹⁸. To determine whether transfected M1 cells were dying through apoptosis, these parameters were investigated. Cells expressing the Val 135 mutant at 32.5 °C displayed a typical apoptotic morphology (Fig. 3a) with a fragmented nucleus and chromatin condensation^{18,19,22}. Furthermore, activation of wild-type p53 (32.5 °C) gave a characteristic DNA 'ladder' (Fig. 3b). The loss of viability at 32.5 °C was partially inhibited by cycloheximide (data not shown), as found for apoptosis in various cell systems^{18,19}. The death elicited by wild-type p53 can thus be described as apoptosis.

Interleukin-6 (IL-6) induces M1 cell differentiation^{5,13,23,24}; in clone S6, the end product is monocytes¹¹. The effect of IL-6 on transfected M1 clones was therefore investigated. At 37.5 °C, such transfectants differentiated in the same way as parental M1 (data not shown). At 32.5 °C, IL-6 also induced myeloid differentiation (data not shown), but IL-6 also markedly reduced

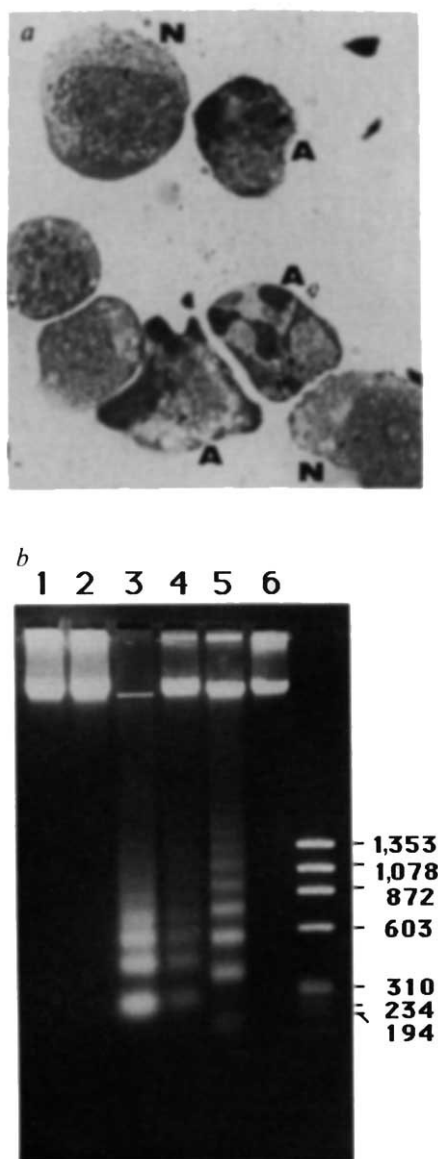


FIG. 3 Apoptotic features of p53-mediated cell death. *a*, Cell morphology. Cells of clone LTR-13 were maintained at 32.5 °C for 12 h, and subsequently fixed, stained with May-Grunwald-Giemsa stain and photographed at a magnification of $\times 1,000$. The panel shows apoptotic (A) and non-apoptotic (N) cells. *b*, DNA fragmentation in cells undergoing p53-mediated cell death. Experimental conditions were essentially as described in ref. 20. Lanes: 1, parental M1 cells, 32.5 °C; 2, LTR-13, 37.5 °C; 3, LTR-13, 32.5 °C; 4, LTR-13 + IL-6 (see Fig. 4), 32.5 °C; 5, SV-1, 32.5 °C; 6, LTR-Phe, overexpressing the constitutive Phe132 p53 mutant, 32.5 °C. Concentrations of mutant p53 in LTR-Phe are comparable to those of LTR-13 (data not shown). Viability values for the samples shown in the individual lanes were as follows: 1, 93%; 2, 94%; 3, 18%; 4, 71%; 5, 52%; 6, 95%.

METHODS. Two million cells were treated variously for 20 h as indicated above. Cells were washed twice in a solution of 140 mM NaCl, 20 mM EDTA, and incubated at 37.5 °C for 4 h in 400 μ l lysis buffer (200 mM Tris-HCl, pH 8.3, 100 mM EDTA, 50 μ g ml⁻¹ proteinase K, 1% SDS). The DNA was extracted with an equal volume of phenol, and the aqueous phase was dialysed overnight against a solution of 10 mM Tris-HCl, pH 7.5, 1 mM EDTA. The DNA solution was incubated for 3 h at 37 °C with 50 μ g ml⁻¹ RNase A, and proteinase K then added to 120 μ g ml⁻¹ and incubation continued for another 3 h. The DNA was then extracted with phenol/chloroform and precipitated in ethanol. Pellets were resuspended in 50 μ l H₂O, and DNA concentration determined from the absorbance at 260 nm. Each DNA sample (3 μ g) was electrophoresed through a 1.5% agarose gel. DNA bands were visualized by staining with ethidium bromide. Sizes in base pairs of molecular size markers (M, *Hae*III digest of double-stranded ϕ X174 DNA) are indicated on the right.

cell death (Fig. 4a); this was correlated with a large reduction in DNA fragmentation (Fig. 3b, lane 4). At 37.5 °C, on the other hand, IL-6 caused a slight loss of viability (Fig. 4b). Hence, IL-6 can inhibit apoptosis mediated by wild-type p53. Our data indicate that restoration of wild-type p53 expression can cause M1 cells to undergo apoptosis, and that IL-6 can promote cell survival under these conditions. Apoptosis clearly requires the wild-type activity of the protein, because it is not elicited by the Phe 132 non-temperature-sensitive mutant of p53 (Fig. 4a), which behaves as a mutant at 32.5 °C¹⁰. Expression of p53 is absent in many human myeloid leukaemic cell lines and in primary cells from leukaemic patients²⁵⁻²⁷. Normally *in vivo* myeloid progenitor cells might continuously die by apoptosis unless the appropriate differentiation or proliferation signal is present. This would keep the number of circulating cells low until they are required more abundantly. If wild-type p53 is involved in this apoptosis, then loss of p53 could relieve this apoptotic programme so that a pretransformed, neoplastic population is generated, where the cells do not die but keep cycling until the accessory oncogenic activation. This mechanism could account for the development of myeloid leukaemia. Inactivation of wild-type p53 does not necessarily require the loss of the p53 allele. In solid tumours, wild-type p53 inactivation occurs most often by point mutations in the coding region^{1,2,28,29}, giving rise to mutant proteins that do not have antiproliferative effects^{10,30-36}. Thus those leukaemic cells that express p53 could in fact be expressing a mutant form.

The proposed involvement of wild-type p53 in mediating apoptosis in the absence of appropriate differentiation or proliferation signals has its parallels. The product of the *bcl-2* oncogene can block the apoptotic death of haematopoietic progenitor cells of different lineages³⁷⁻³⁹, and the Epstein-Barr virus has a similar effect on B cells⁴⁰. Thus, whereas most oncogenes probably contribute to neoplasia by promoting cell proliferation, cancer may also be induced through the activation of genes whose products allow the survival of a cell that should otherwise die. So if tumour suppressor genes mediate apoptosis, their inactivation will have the same effect.

So far there is no evidence that p53 is a mediator of apoptosis

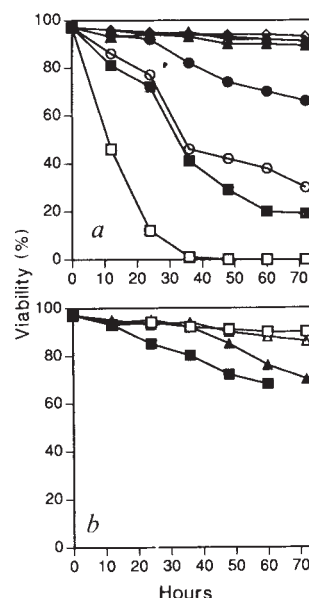


FIG. 4 Inhibition of p53-mediated cell death by IL-6. Cells of various M1-derived clones were maintained at either 32.5 °C (*a*) or 37.5 °C (*b*) without (open symbols) or with (filled symbols) IL-6. Recombinant human IL-6 (Interpharm, purified to 1.6×10^7 U mg⁻¹) was added to a final concentration of 8 ng ml⁻¹ at time 0; for *a*, this represented the time at which the cells were transferred to 32.5 °C. Clones used: SV-1 (circles); LTR-13 (squares); LTR-Phe (diamonds); neo, transfected with pSV2neo alone (triangles).

in normal cells. Yet, such a role might represent an additional link between tumour suppressor genes and carcinogenesis. □

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Bovine papillomavirus E5 oncoprotein binds to the 16K component of vacuolar H⁺-ATPases

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THE major transforming protein of bovine papillomavirus type 1, E5 (refs 1–4), is mainly associated with endomembranes^{5,6}, specifically binding to a cellular protein of relative molecular mass 16,000 (16K) (ref. 7). At the same time as transformation, E5 causes the phosphorylation of tyrosine residues in epidermal and platelet-derived growth factor receptors^{8,9}. We show here that the 16K protein associated with E5 is the 16K component of vacuolar ATPases. This protein is known to be an integral membrane protein in endosomes, bovine chromaffin granules, synaptic vesicles, fungal and plant vacuoles and clathrin-coated vesicles^{10–16}, as well as a component of gap-junction-like membrane complexes¹⁷. Because proton pumps are critical for the function of cellular compartments that process growth-factor receptors, the interaction of E5 with the 16K protein could explain the pleomorphic features of cells transformed by E5.

Considerations of size, hydrophobicity, cell localization and lack of cysteine residues suggested that the 16K component of the F₀-like proton channel of vacuolar H⁺-ATPases might be the E5-associated 16K protein. To evaluate this possibility, rabbit antiserum generated against the chicken-liver vacuolar 16K protein (which cross-reacts with 16K vacuolar proteins from mammalian and arthropod tissues¹⁸) was used in a series of immunoprecipitation experiments.

The PL15 construct was used to express high levels of an epitope-E5 fusion protein in permissive monkey cells¹⁹ and has been used previously to detect the E5-associated 16K protein

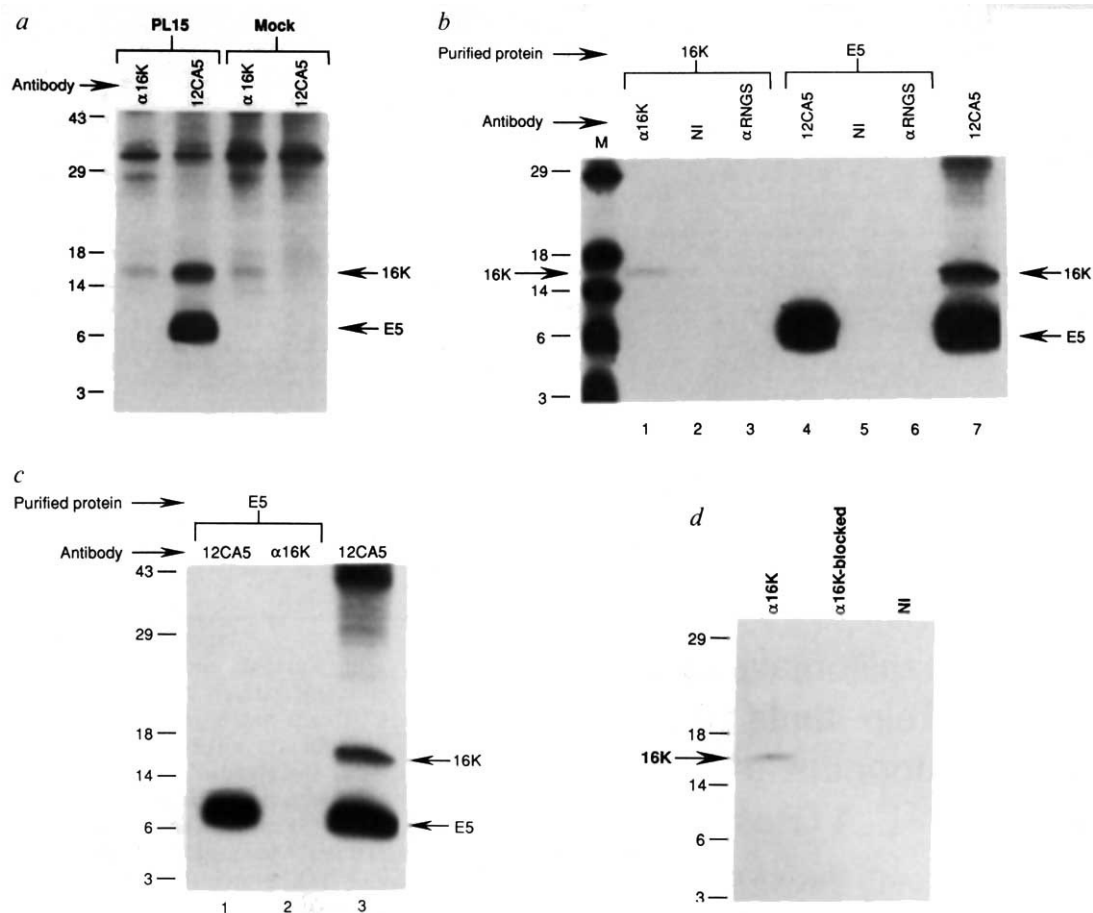
using a monoclonal antibody (12CA5) that recognizes the HA1 epitope⁷. Extracts were prepared from [³⁵S]methionine-labelled COS cells which were either mock-infected or infected with the recombinant virus PL15. Proteins immunoprecipitated with either the anti-16K or 12CA5 antibody migrate identically on 15% polyacrylamide gels (Fig. 1a). Anti-16K antibodies precipitate a 16K protein from both mock-infected and PL15-infected extracts, whereas 12CA5 precipitates only E5 and the E5-associated 16K protein from PL15-infected cell extracts. Anti-16K antibodies do not co-precipitate E5 from extracts of PL15-infected cells, indicating that either the epitopes recognized by these antibodies are blocked by the interaction with E5 or that insufficient amounts of 16K were precipitated to detect E5. Previous experiments had shown that the 12CA5 antibody does not interact directly with the E5-associated 16K protein⁷.

To show that anti-H⁺-ATPase 16K antibodies can precipitate purified E5-associated 16K protein, [³⁵S]methionine-labelled, PL15-infected COS cells were immunoprecipitated with affinity-purified 12CA5 antibody. The immunoprecipitated E5/16K complex was separated by PAGE and visualized by autoradiography. The E5 and 16K proteins were individually purified from gel slices by electroelution and 16K immunoprecipitated with anti-16K, 12CA5, nonimmune, or αRNGS antiserum²⁰ (generated against an irrelevant epitope) (Fig. 1b). Lane 1 shows that H⁺-ATPase 16K antibodies reacted specifically with the purified E5-associated 16K protein. No 16K protein was seen using nonimmune or αRNGS antiserum as a negative control (lanes 2 and 3). As a positive control, purified epitope-E5 protein was incubated in the same fashion with 12CA5, nonimmune and αRNGS antiserum. Whereas 12CA5 precipitated large amounts of purified E5 protein (lane 4), no E5 was evident when nonspecific antibodies were used (lanes 5, 6). When anti-16K antibodies were incubated with equivalent amounts of E5 protein, no E5 protein was detected (Fig. 1c) demonstrating the specificity of the 16K antibody. Finally, when H⁺-ATPase-specific 16K antibodies were pre-absorbed with 16K protein purified from *Nephrops norvegicus* (Norway lobster)^{18,21}, immunoprecipitation of purified 16K protein was nearly totally abolished (Fig. 1d, lane 2) compared to non-absorbed serum (Fig. 1d, lane 1). These data provided strong evidence that the E5-associated 16K protein is the vacuolar H⁺-ATPase 16K protein.

It has been shown previously that the 16K protein associated with the E5 oncoprotein contains methionine but no cysteine residues⁷. The deduced sequence of the *Drosophila* vacuolar 16K protein also contains methionine but not cysteine²². We

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FIG. 1 Immunoprecipitation of 16K proteins using anti-sera raised against chicken liver vacuolar ATPase 16K protein. Extracts or purified proteins were immunoprecipitated using the indicated antibodies. Immunoprecipitated proteins were electrophoretically separated by 15% SDS-PAGE and visualized by fluorography. *a*, Comigration of E5-associated and vacuolar ATPase 16K proteins. Extracts were prepared from PL15- or mock-infected COS cells metabolically labelled with [35 S]methionine (NEN Expre 35 S Protein Labelling Mix) as described previously⁷ and immunoprecipitated using the indicated antibody. Positions of the 16K and E5 proteins are indicated on the right and molecular weight markers (K) on the left. *b*, Immunoprecipitation of purified E5-associated protein with vacuolar-ATPase 16K antibodies. Metabolically labelled, PL15-infected COS cell extracts were immunoprecipitated with affinity-purified 12CA5 ascites fluid. Following separation by SDS-PAGE, E5-associated 16K protein and E5 protein were purified by electroelution using a Bio-Rad electroelutor, and re-immunoprecipitated using the antibodies indicated. Lane 7 shows a 12CA5 immunoprecipitation of extracts from PL15-infected COS cells from which the E5 and 16K proteins were purified. After electroelution into SDS-PAGE running buffer (0.25M Tris-HCl, pH 8.3, 0.192M glycine, 0.1% SDS), isolated proteins were diluted 10-fold with a modified RIPA buffer and incubated with antibodies overnight at 4 °C. Protein-antibody complexes were isolated with protein A-Sepharose CL4B beads (Pharmacia Inc., Piscataway, NJ) and prepared for electrophoresis as described⁷. Positions of 16K and E5 proteins are indicated. Purified 16K protein (lanes 1–3) was re-immunoprecipitated with 40 μ l anti-16K serum, 40 μ l nonimmune (NI) serum, and 5 μ l of α RNGS antiserum, which is a



high-titre polyclonal antiserum against an epitope of the lymphocyte-specific protein kinase, *lck*²⁰. Purified E5 protein was immunoprecipitated with 5 μ l 12CA5, 40 μ l nonimmune serum (NI) and 5 μ l α RNGS serum. *c*, Antiserum to vacuolar-ATPase 16K protein does not react nonspecifically with any hydrophobic protein. E5 protein, purified by immunoprecipitation with 12CA5, SDS-PAGE and electroelution, was incubated overnight with either 5 μ l affinity purified 12CA5 or 40 μ l vacuolar-ATPase 16K protein antiserum. *d*, Specificity of the E5-associated 16K protein precipitation with anti-vacuolar-ATPase 16K protein antiserum. The 16K serum was used either unmodified or pre-absorbed (blocked) with purified lobster 16K protein^{18,21} to precipitate purified E5-associated 16K protein.

a

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1  ATG TCC GAG GCC AAG AAC GGC CCC GAG TAC GCT TCC TTT TTC GCG 45
   M S E A K N G P E Y A S F F A

46  GTC ATG GGT GCC TCA GCC GGC ATG GTC TTC AGC GCC CTT GGC GCC 90
   V M G A S A A M V F S A L G A

91  GCC TAC GGT ACA GCC AAG AGC GGC ACG GGC ATC GCA GCC ATG TCT 135
   A Y G T A K S G T G I A A M S

136 GTC ATG CGG CCA GAG ATG ATC ATG AAG TCC ATC ATC CCG GTG GTC 180
   V M R P E M I K S I I P V V

181 ATG GCG GGG ATC ATC GCC ATC TAT GGT CTG GTG GTG GCA GTC CTC 225
   M A G I V I A I Y V A I V L

226 ATT GCC AAC TCC CTG AAT GAC GGC ATC AGT CTC TAC AGG AGT TTC 270
   I A N S L N D G I S L Y R S F

271 CTT CAG CTG GGC GCA GGC TTG AGT GTG GGC CTG AGC GGG CTG GCG 315
   L Q L G A G L S V G L S G L A

316 GCA GGC TTC GCC ATC GCC ATT GIT GGG GAC GCA GGC GTG CGT GCC 360
   A G F A I G I V G D A G V R G

361 ACC GCC CAG CAG CCG CGG CTC TTC GTG GGC ATG ATC CTC ATC CTC 405
   T A Q Q C P R L F V G M I L I L

406 ATC TTC GCC GAG GTG CTC GGC CTC TAC GGT CTC ATC GTC GCC CTT 450
   I F A E V L G L Y G L I V A L

451 ATC CTC TCC AAG TAG 465
   I L S K *
  
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b

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A A G G F A I G I V G D
G C G G C A G G C T T C G C C A T C G G C A T T G T T G G G G A C
313-: : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
g c g g c a c g t t c s c a t c s g a c a t l g t t g g g a c
A A R S P S G A L L G G T

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A G G V R G C T A
G C A G G C G T G C A C C G C C
: : : : : : : : : : : : : : : : : : : : : : : : : : : : : : : :
g c a g g c g t g c g t g c a c c g g c
C G R A C T A

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FIG. 2 Sequence of a bovine cDNA coding for the vacuolar 16K protein. A commercial cDNA library from bovine adrenal medulla tissue (Stratagene) was screened with oligonucleotides corresponding to the 3'-untranslated regions of the published sequence for the 16K protein of the vacuolar H⁺-ATPase¹⁰. A single cDNA of 0.8 kilobase (kb) was isolated and sequenced in pIC-20H (double stranded) and M13 vectors (single-stranded). *a*, The sequence of the entire bovine 16K open reading frame. *b*, DNA and corresponding amino-acid sequences of the region (nucleotide 313–366) of difference between this study and the cloning study of Mandel *et al.*¹⁰. Upper-case letters, nucleotide sequence along with the deduced amino-acid sequence (single-letter code) of the cDNA isolated in this study; lower-case letters, original nucleotide sequence with the corresponding amino-acid sequence of ref. 10. No other differences were found between the two sequences.

chose to clone and sequence the bovine form of the vacuolar 16K protein because an earlier reported sequence¹⁰ of a bovine cDNA showed a single cysteine residue in a stretch of 14 amino acids which had no identity with the equivalent, conserved region of the 16K vacuolar protein from other eukaryotes (yeast, *Nephrops novaeigicus*, *Drosophila* and *Torpedo*)²²⁻²⁴. Sequencing of the bovine cDNA (Fig. 2) demonstrates the incorrect assignment of these 14 amino acids in the original sequence and reveals that the bovine 16K protein also lacks cysteine (Fig. 2b). Further evidence for the lack of cysteine residues in mammalian forms of the vacuolar 16K protein is the inability of the mouse protein to be labelled with [¹⁴C]iodoacetic acid (data not shown).

Finally, a proteolytic digestion of purified 16K proteins was performed using V8, lys-C and alkaline proteases^{25,26}. The 16K protein from lobster hepatopancreas and [³⁵S]methionine-labelled 16K protein translated *in vitro* using the bovine 16K cDNA were proteolytically cleaved following gel purification and compared to cleavage patterns of gel-purified, E5-associated 16K protein. All 16K proteins were resistant to all enzymes assayed except for alkaline protease which cleaved E5-associated 16K and H⁺-ATPase 16K proteins into two fragments (12K and 4K) (Fig. 3). These digestion patterns confirm the conclusions of the antibody-precipitation studies that the E5-associated 16K protein is the major 16K component of the vacuolar H⁺-ATPases and gap-junction-like membrane complexes.

E5-transformed cells show constitutive activation of cell receptors for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). E5 expression stimulates the phosphorylation of the EGF receptor in a ligand-independent manner and decreases the down-regulation of these occupied receptors⁸. Similarly, E5 also stimulates the phosphorylation of the PDGF receptor⁹. One potential site for E5 action would be

the endosomal compartment. Vacuolar H⁺-ATPases generate an acidic internal endosomal pH which is critical for dissociating ligand-receptor complexes as well as targeting these complexes for lysosomal degradation. Interference with this proton pump by the binding of E5 to the 16K protein might result in prolonged growth factor/receptor interactions and recycling of receptors to the cell surface. Although this mechanism of action might explain the activation of cell surface receptors and subsequent mitogenesis, alternative targets for E5 such as the Golgi apparatus or gap junctions may be equally important for its biological activity. □

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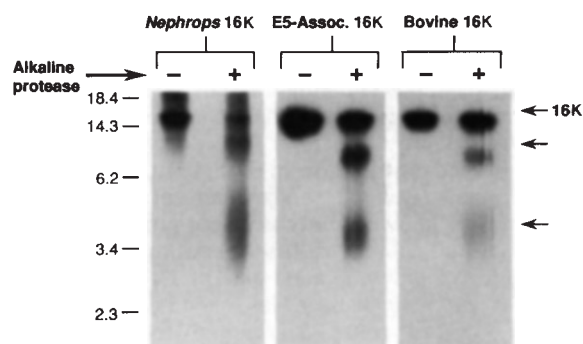


FIG. 3 Partial proteolytic cleavage of *Nephrops*, bovine and E5-associated 16K proteins. Purified 16K proteins were subjected to partial proteolytic cleavage using alkaline protease (Promega, Madison). Briefly, E5-associated 16K protein was purified from metabolically-labelled, PL15-infected COS cell extracts by immunoprecipitation with 12CA5 ascites fluid. 12CA5-precipitated E5/16K complex was separated by SDS-PAGE and visualized by autoradiography. The bovine 16K cDNA (see Fig. 3 legend) was cloned into the pTZ18R vector (Bio-Rad) and transcribed *in vitro* using T7 RNA polymerase. RNA was used to synthesize [³⁵S]methionine-labelled 16K protein *in vitro* using a wheat-germ extract (Promega). *In vitro* synthesized 16K protein was separated by SDS-PAGE and visualized by autoradiography. *Nephrops* vacuolar-ATPase 16K protein, purified from hepatopancreas as described^{18,21}, was separated by SDS-PAGE, visualized for 10 min with 0.25% Coomassie brilliant blue G-250 (Bio-Rad) in 25% isopropanol, 10% glacial acetic acid and destained for 2-3 h with water. Gel slices containing 16K proteins were subjected to proteolysis with 5-50 µg ml⁻¹ alkaline protease and proteolytic fragments were separated by electrophoresis using 20% SDS-PAGE. Proteolytic fragments of the E5-associated 16K and *in vitro* synthesized, bovine 16K proteins were visualized by fluorography, whereas fragments of the vacuolar-ATPase 16K protein were visualized by silver staining. Positions of cleaved and uncleaved 16K proteins are indicated by arrows to the right of the gel. Relative molecular mass markers (K) are indicated to the left.

Was the loss of the D helix in α globin a functionally neutral mutation?

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PROTEINS in the globin family are found in a variety of species from bacteria to man¹⁻³. From the many globin sequences known, evolutionary trees have been constructed showing that α and β globins diverged from a common ancestor between 425 and 500 million years ago, after vertebrate species had appeared and roughly when sharks and bony vertebrates diverged⁴⁻⁶. The α and β globins assemble to form tetrameric haemoglobin, $\alpha_2\beta_2$, which can switch between quaternary states having high and low oxygen affinity⁷. This allows the protein to bind oxygen cooperatively and therefore efficiently transport oxygen from the lungs to respiring tissues. The α and β globins have closely related tertiary structures, being α -helical proteins with similar haem-binding sites. Most globins consist of eight helices, designated A to H from the

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FIG. 1 Amino-acid sequence alignment of part of the human α and β globins. In α globin, the residues corresponding to residues 50–54 in β globin are missing. This missing sequence is called the Braunitzer gap⁴ and corresponds to the D helix. The same region is missing from the β globin of the dogfish shark *Squalus acanthias* and the Port Jackson shark *Heterodontus portijacksoni*³.

	C helix	40	CD	50	E helix
α globin	Lys-Thr-Tyr-Phe-Pro-His-Phe-		-Asp-Leu-Ser		His-Gly-Asn-Pro
β globin	Gln-Arg-Phe-Phe-Glu-Ser-Phe-Gly-Asp-Leu-Ser-Thr-Pro-Asp-Ala-Val-Met-Gly-Ser-Ala				
	C helix	40	CD	50	D helix

N terminus, connected by short nonhelical segments, but all known vertebrate α globins lack a D helix. Because the loss of this helix by α globin occurred shortly before tetrameric haemoglobin appeared, it might be a functionally important mutation required for a tetramer assembly or allostery. We have now tested this idea by engineering human haemoglobins containing β subunits without a D helix and α subunits with a D helix. Both of these mutations have little effect on the oxygen-binding properties of the molecule. Thus it is possible that deletion of the D helix in the α subunit was caused by a neutral mutation⁸.

Amino-acid sequence alignments of vertebrate α and β globins show that α globins are missing five residues equivalent to residues 51–55 in human β globin^{1–3} (Fig. 1). Human α globin has 141 amino-acid residues, whereas human β globin has 146. The crystallographic structure of haemoglobin (Hb) shows that these missing residues correspond to part of the D helix in β globin⁷ (Fig. 2). This pattern is conserved in the globin sequences of all bony vertebrates, even though the overall sequence identity of the most distantly related vertebrate Hbs is only 40% (ref. 3). The crystal structure of Antarctic fish Hb confirms this conservation (G. Fermi, M. F. Perutz and G. di Prisco, unpublished results). Crystal structures have been determined for globins from the root nodule of the lupin *Lupinus leuteus*⁹, the larva of the midge *Chironomus thummi*¹⁰, the sea lamprey *Petromyzon mainus*¹¹, the marine bloodworm *Glycera dibranchiata*¹², the clam *Scapharca inaequivalvis*¹³, sperm whale¹⁴, pig¹⁵, horse⁷ and man⁷. All of these globins contain a D helix, except the α subunit of the Hbs of the vertebrates *Glycera* and *Scapharca*. The amino-acid sequence of globin from the bacterium *Vitreoscilla* indicates that it may also contain the D helix¹⁶. It is not immediately clear from the structure of Hb whether or not this is functionally significant. We synthesized two Hb mutants, one by removing the D helix from β globin (' βD^- ') and one by inserting a D helix into α globin (' αD^+ '). Oxygen equilibrium curves were then obtained for these mutants.

Hb was expressed in *Escherichia coli* using the vector developed by Hoffman *et al.*¹⁷ which carries both human α and β globin genes under the control of the *Tac* promoter, and produces soluble, folded, functional Hb. The Met residues encoded by the initiation codon remain attached to the N termini of both the α and β subunits as in most foreign proteins expressed in *E. coli*. Therefore this vector has been modified by deleting the first (valine) codon of each gene. Because the

initiation methionine residue is not cleaved from the N terminus, our control 'wild-type' Hb is identical to normal human 'wild-type' Hb (HbA₀) except for two mutations, Val 1 α $\rightarrow$ Met and Val 1 β $\rightarrow$ Met. Its oxygen affinity and cooperativity are slightly lower than those of HbA₀ (D.L., J. Miller and G. Stetler, unpublished results). The expression vectors for βD^- and αD^+ Hbs were constructed by cassette mutagenesis. Both clones produced soluble, functional Hbs despite the insertion or deletion of the helix. This indicates that both α and β chains are produced, folded and assembled into tetramers *in vivo* because large amounts of soluble Hb accumulate in the cells only when α and β chains are coexpressed¹⁷. We demonstrated that both αD^+ and βD^- Hbs consist of α and β subunits by reverse-phase chromatography of the proteins on a Vydac C₄ column¹⁷.

Figure 3 shows the Hill plots of oxygen-binding curves of the mutant and control 'wild-type' Hbs. P_{50} is the value of the partial pressure of (pO_2) at which 50% of Hb is bound to oxygen and is used as a measure of overall oxygen affinity. In Fig. 3 P_{50} is the pO_2 value at the intercept on the $\log [Y/(1-Y)] = 0$ axis. The oxygen equilibrium curve of αD^+ Hb is almost identical to that of 'wild-type' Hb, and deletion of the D helix in the β subunit only slightly increases the oxygen affinity of the protein. The Hill coefficient, n , is the slope of the Hill plot at 50% saturation and is a useful measure of the extent of cooperativity between the haems. The Hill coefficient is unchanged by these mutations, indicating that the allosteric transition on oxygen binding, and therefore the intersubunit contacts, are not substantially altered by these mutations. Clearly the D helix is not required for tetramer assembly and plays no great part in regulating the oxygen affinity.

The widespread distribution of D helix-containing globins throughout plants, bacteria and animals suggests that the D helix was present in a very early ancestral protein but was lost in the α globin of vertebrates. It is interesting to note that the dogfish shark *Squalus acanthias* and the Port Jackson shark *Heterodontus portijacksoni*, have Hbs that do not have a D helix in either the α or β subunits¹⁸. *S. acanthias* Hb is mainly dimeric when oxygenated.

It is known from the many clinically detected mutations in human Hb that mutations at the protein surface rarely affect its physiological properties¹⁹. The βD^- helix is not involved in any intersubunit contacts, unlike the C helix which packs against the FG helix corner of an α subunit; nor does it seem to be involved in switching between liganded and unliganded states

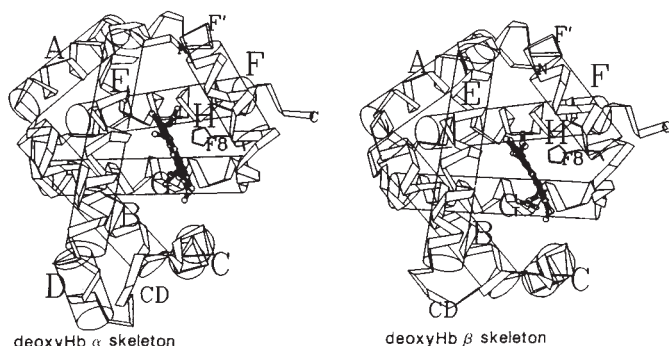


FIG. 2 Ribbon diagrams of the subunits of human deoxyhaemoglobin. The α helices are indicated by cylinders; β globin has eight helices, A to H, but α globin has only seven. The haem group to which oxygen can bind is also shown; it lies between the E and F helices. Ligands binding to the haem lie in van der Waals' contact with two residues of the E helix, histidine E7 and valine E11 (not shown). The histidine residue (F8) that forms a covalent bond to the haem iron atom is marked.

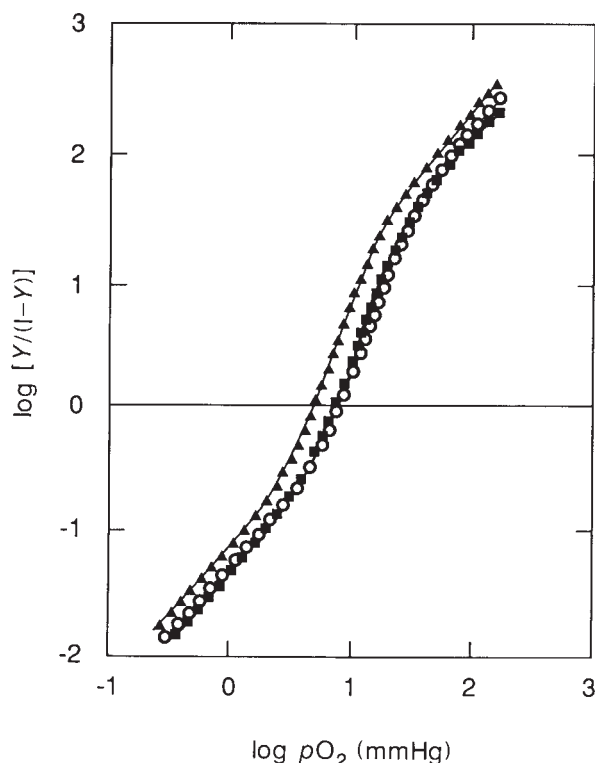


FIG. 3 Hill plot of oxygen equilibrium curves of wild-type, αD^+ and βD^- Hbs. $\circ$, Wild-type Hb made by the method of Hoffman *et al.*⁹; $\blacksquare$, αD^+ Hb; $\blacktriangle$, βD^- Hb. Y, fractional saturation of Hb with oxygen; pO_2 , partial oxygen pressure in mmHg. The Hill plot reaches an asymptote with a slope of 1 at both very high and very low pO_2 . The lower asymptote cuts the $\log[Y/(1-Y)] = 0$ axis at a pO_2 value corresponding to the value of K_T , the oxygen association constant of the T-state protein. Similarly, the upper asymptote cuts the axis at a pO_2 value corresponding to the value of K_R , the oxygen association constant of the R state protein. Both of the mutant proteins have K_R and K_T very close to the values found for HbA. This indicates that the mutants can form a T-state structure very similar to that of HbA because the oxygen affinities of the α and β subunits of Hb are lowered by different mechanisms in the T state conformation. Cooperativity requires the subunit contacts to change as oxygen binds, and is very sensitive to mutations that alter the relative stability of either quaternary state. The D helix mutations do not affect n , and so the intersubunit contacts do not appear to be disturbed.

METHODS. The expression vector is identical to that described in ref. 17 except that the codons for the first Val residue of the α and β subunits have been deleted (D.L., J. Miller and G. Stetler, unpublished results). The 'wild-type' Hb has the Val 1 α $\rightarrow$ Met, Val 1 β $\rightarrow$ Met mutations and shows slightly reduced oxygen affinity and cooperativity relative to human adult HbA₀ from human blood. For expression of αD^+ Hb, residues Thr-Pro-Asp-Ala-Val were inserted between residues 49 and 50 by cassette mutagenesis. βD^- Hb has the deletion of residues between residues 50 and 54. *E. coli* cells were grown in 2 $\times$ TY medium in the presence of 100 μ M ampicillin and carbon monoxide for 24 h at 37 $^\circ$ C. Cells were collected by centrifugation and resuspended in 150 ml of 10 mM sodium phosphate buffer, pH 6.0, saturated with carbon monoxide and lysed by sonication. Hb was purified by the method of B. Vallone, N.H.K. and K.N. (unpublished results) and described briefly below. Cell debris were removed by centrifugation at 35,000 r.p.m. in Beckman 70Ti rotor and the pH of the supernatant was adjusted to 6.0 with 1 M phosphoric acid. The crude extract was applied to a 6 $\times$ 15-cm CM-cellulose column equilibrated with 10 mM sodium phosphate buffer, pH 6.0, and Hb was eluted with a linear gradient as described in ref. 21. Hb was concentrated using Amicon PM10 membrane, and oxygen equilibrium curves were obtained using the automatic recording apparatus described in ref. 22.

like the E helix, which lies across the ligand-binding face of the haem⁷. The role of the D helix may simply be to link the C and E helices. Our experiment shows that these two helices can be linked with the flexible CD segment just as readily with or without the D helix, without affecting the oxygen-binding properties. Some naturally occurring human Hb mutants have amino-acid replacements in the CD segment, some of which result in altered oxygen affinity or instability¹⁹. It is, therefore, remarkable that the loss or addition of the D helix has such a small effect on oxygen-binding properties and stability.

The β chains form homotetramers with a quaternary structure similar to that of R state (liganded) Hb. The β_4 homotetramer binds oxygen noncooperatively and its oxygen affinity is too high to unload oxygen effectively in the tissues. The α chains bind oxygen noncooperatively with similar affinity to that of the β_4 homotetramer. Only when the α and β chains are assembled into $\alpha_2\beta_2$ tetramers can they undergo allosteric transition between two alternative quaternary structures: the T structure

with low oxygen affinity and the R structure with high oxygen affinity. Assembly into $\alpha_2\beta_2$ tetramers was therefore a crucial step in Hb evolution. Our experiment shows that the presence or absence of the D helix does not affect assembly into cooperative tetramers. Therefore assembly into $\alpha_2\beta_2$ tetramers and the loss of the D helix are likely to have been independent events in Hb evolution. The loss of the D helix in the α subunit may have been caused by a neutral mutation which became fixed at an early stage of vertebrate Hb evolution. Alternatively its loss may have had some selective advantages when α and β globins diverged or the α and β globins were first assembled into primitive allosteric dimers or tetramers. In fact the haem-haem interaction arises from different molecular mechanisms²⁰ in lamprey¹¹ and *Scapharca* Hbs¹³. At a later stage when additional mutations gave rise to more sophisticated allosteric mechanisms, this selective pressure may have become insignificant so that the loss of the D helix now seems to be the result of a neutral mutation in modern vertebrate Hbs. $\square$

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Sliding distance between actin and myosin filaments per ATP molecule hydrolysed in skinned muscle fibres

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MUSCLE contraction is generally thought to be driven by tilting^{1,2} of the 19-nm-long myosin head³, part of the thick filament, while attached to actin, part of the thin filament. This motion would produce about 12 nm of filament sliding^{4,5}. Recent estimates of the sliding distance per ATP molecule hydrolysed by actomyosin *in vitro* vary widely from 8 nm (ref. 6) to ≥ 200 nm (ref. 7). The latter value is incompatible with a power stroke incorporating a single tilting motion of the head. We have measured the isotonic sliding distance per ATP molecule hydrolysed during the interaction between myosin and actin in skinned muscle fibres. We directly estimated the proportion of simultaneously attached actomyosin complexes and their ATP use. We report here that at low loads the interaction distance is at least 40 nm. This distance corresponds to the length of the power stroke plus the filament sliding while actomyosin crossbridges bear negative drag forces^{5,8}. If the power stroke is 12 nm, then our results indicate the drag distance to be at least 28 nm. Our results could also be explained by multiple power strokes per ATP molecule hydrolysed.

Previous studies^{6,7,9,10} of the actomyosin sliding distance with myofibrils and with *in vitro* assays of motility used indirect estimates for the number of myosin heads interacting with the actin filaments and the number of ATP molecules hydrolysed, both crucial parameters. Here, mechanical stiffness of the skinned muscle fibres was monitored during sliding to estimate the number of thick and thin filament sites simultaneously interacting¹¹. Laser-pulse photolysis of caged ATP in the fibres immersed in paraffin oil supplied the contractile apparatus with known and limited amounts of ATP. The number (N_A) of ATP molecules hydrolysed per myosin head in the fibre can be calculated from the concentration ($[ATP_u]$) of ATP used by actomyosin divided by the total concentration ($[M_0]$) of myosin heads in the fibre; $N_A = [ATP_u]/[M_0]$. Interaction distances (D_i) of myosin heads with thin filaments were calculated as

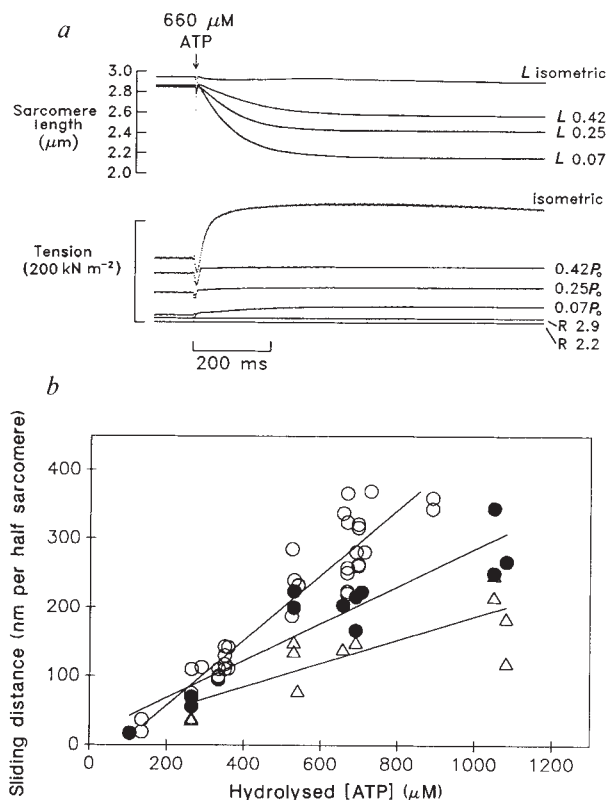
$$D_i = (D_s/N_A)S_a = D_s S_a [M_0]/[ATP_u]$$

where D_s is the total sliding distance determined as shortening per half sarcomere and S_a is the average stiffness relative to rigor at full overlap between the thin and thick filaments. S_a is assumed to indicate the proportion of myosin heads forming actomyosin crossbridges during the contraction. $[M_0]$ was assumed to be 154 μM (ref. 12) as determined under conditions similar to ours.

Single glycerol-extracted fibres from rabbit psoas muscle at a sarcomere length of about 2.8 μm were put into rigor (no ATP), transferred to a rigor solution with about 30 μM free Ca^{2+} (Ca^{2+} -rigor), and then to photolysis solution, which was Ca^{2+} -rigor plus caged ATP, a photolabile precursor of ATP. The fibre was transferred into paraffin oil in a trough with fused-silica windows for photolysis and for measurement of sarcomere length. The concentration of ATP released by photolysis and hydrolysed by the fibre was determined by high-

FIG. 1 Isotonic shortening of a skinned muscle fibre after photoliberation of ATP. **a**, Sarcomere length (upper traces) and tension (lower traces) when 660 μM ATP was released by photolysis of caged ATP. The recordings were made in isometric conditions and at 0.07, 0.25 and 0.42 of isometric tension (P_0) as indicated next to each tension and sarcomere length (L) trace. R 2.2 and R 2.9 are base line tensions at 2.2 and 2.9 μm sarcomere length. All recordings were filtered through 500 Hz notch filters to eliminate imposed sinusoidal oscillations. Initial caged ATP concentration was 2.21 mM. **b**, Total sliding distance versus hydrolysed ATP concentration for a series of fibres. Liberated $[ATP]$ was varied mainly by altering the initial $[caged\ ATP]$. The shortening distance is the difference in half sarcomere length measured before the laser pulse and after exhaustion of the liberated ATP. Mean isotonic tensions were 0.07 ($\circ$), 0.25 ($\bullet$) and 0.46 ($\triangle$) P_0 . The lines were fitted by least-squares regression.

METHODS. Preparation of the fibres and the apparatus are essentially as described before¹⁴. Glycerol-extracted single fibres from rabbit psoas muscle were mounted horizontally to hooks on a tension transducer and a linear moving-coil motor¹³ in relaxing solution. The fibre was stretched to a sarcomere length of about 2.8 μm and treated briefly with 0.5% Triton X-100 to remove residual membrane components. To measure sliding distance, the fibre was put into rigor, washed with Ca^{2+} -rigor solution and finally Ca^{2+} -rigor solution with caged ATP. This photolysis solution contained (mM): TES buffer, 100; reduced glutathione, 10; calcium-EGTA, 53; free Ca^{2+} , 0.03; free Mg^{2+} , 1; AP_5A (diadenosine pentaphosphate), 0.04; caged ATP (P^{3-1} -(2-nitro)phenylethyladenosine 5'-triphosphate), 0.45–3.62 mM; ionic strength, 200; pH, 7.1; 19–21 °C. The fibre was transferred to a fused silica trough containing paraffin oil for caged ATP photolysis triggered by a 50 ns, 347 nm pulse from a frequency-doubled ruby laser¹⁴ and for measurement of sarcomere length by white-light diffraction¹⁵. For calibration of the hydrolysed ATP concentration versus the laser energy, the fibre was incubated in the Ca^{2+} -rigor solution with about 2 mM 3H -labelled caged ATP and activated by photolysis in oil. After about 1 min, the radioactivity was washed out into relaxing solution containing (mM) Mg^{2+} -ATP, 1; caged ATP, 0.2; ADP, 0.2 and AMP, 0.1. Caged ATP and nucleotides in the washout solution were separated by HPLC and the radioactivity in each peak was determined by liquid scintillation counting¹². The ratio of liberated 3H -labelled ADP (from photoliberated 3H -labelled ATP) to the initial concentration of 3H -labelled caged ATP increased linearly with laser energy.



performance liquid chromatography (HPLC) separation and scintillation counting of radioactive caged ATP and nucleotides. At 7–20 ms after the photolysis of caged ATP by a laser pulse, tension generated by the fibre was clamped to a preset value by feedback to a moving-coil motor¹³. The fibre then contracted isotonicly until the ATP was exhausted (Fig. 1a). The shortening velocity decreased gradually during the isotonic phase because the concentration of ATP decreased and ADP accumulated in the fibre. When shortening stopped, the fibre was again in rigor at the shorter length.

Sliding distance per half sarcomere (D_s) decreased as the isotonic load was increased (Fig. 1a) and increased with liberated ATP concentration (Fig. 1b). The initial velocity of shortening, measured 5–20 ms after initiation of the tension clamp, also increased with liberated ATP concentration. The initial velocities were 2.74 ± 0.08 (s.e.m., $n = 21$), 1.54 ± 0.09 ($n = 9$) and 0.75 ± 0.06 ($n = 9$) $\mu\text{m s}^{-1}$ per half sarcomere at isotonic tensions averaging 0.07, 0.25 and 0.47 of isometric tension (P_0), respectively, at $728 \pm 30 \mu\text{M}$ ($n = 39$) ATP. The initial velocity was 1.56 ± 0.12 ($n = 13$) $\mu\text{m s}^{-1}$ per half sarcomere at $0.05 P_0$ and $328 \pm 10 \mu\text{M}$ ATP.

Stiffness of the fibre was measured by detecting the sinusoidal tension and sarcomere length oscillations (Fig. 2a) due to an applied 500 Hz sinusoidal length change of amplitude 1–4 nm per sarcomere peak-to-peak. The sinusoidal components of sarcomere length and tension were passed through digital bandpass filters to reduce noise (Fig. 2b). The observed stiffness (S_{obs}) was calculated as the amplitude ratio of the tension and sarcomere length oscillations relative to that in rigor before photolysis. The stiffness (S_r) relative to the rigor stiffness at full overlap between the thick and thin filaments is given by $S_{\text{obs}}(3.8 - L_i)/(3.8 - 2.4)$, where L_i is the initial sarcomere length in μm , and rigor stiffness is assumed to decrease linearly with sarcomere length between 2.4 and $3.8 \mu\text{m}$ (ref. 16). S_r decreased to about half of its initial value just after the laser pulse (Fig. 2c), indicating myosin head detachment from the thin filaments¹⁴. During the initial period of shortening, the stiffness was reduced at lower loads but then increased as [ATP] decreased and filament overlap increased. On the assumption that the stiffness indicates the proportion of myosin heads simultaneously attached to thin filaments, the average proportion (S_a) of heads attached during sliding, relative to the total number at full overlap, was calculated by averaging stiffness during shortening weighted by the sliding distance in each interval.

The concentration of ATP hydrolysed by actomyosin, $[\text{ATP}_u]$, was calculated by subtracting the concentration ($[\text{M}_H]$) of myosin heads in the H-zone (nonoverlap region of the thick filaments) at the final sarcomere length from the total ATP hydrolysed in the fibre; $[\text{M}_H] = [\text{M}_0](L_f - 2.4)/(3.8 - 2.4)$. This small correction was applied if $L_f > 2.4 \mu\text{m}$, because ATP binds to myosin as rapidly as to actomyosin but the hydrolysis products are not released by myosin during the isotonic shortening period under the present conditions¹².

The sliding distance (D_i) of interaction between actin and myosin per ATP molecule hydrolysed was calculated as $D_s S_a [\text{M}_0]/[\text{ATP}_u]$. D_i decreased from 40 nm to 20 nm when the isotonic tension was increased from 0.07 to $0.46 P_0$ at $[\text{ATP}_u] > 500 \mu\text{M}$ (Fig. 3). D_i is the value of the actomyosin interaction length averaged over the period while the ATP concentration in the fibre fell from its initial value ($> 500 \mu\text{M}$) to zero as the fibre hydrolysed the limited amount available. Toward the end of the shortening phase, actomyosin 'rigor complexes' that have ADP or caged ATP bound or no nucleotide would pose an internal load. Because an external load reduces D_i (open circles in Fig. 3), internal loads are also expected to reduce D_i . This notion is supported by the lower D_i values obtained when the $[\text{ATP}_u]$ was lowered and/or ADP was added to the photolysis solution (Fig. 3). The values of D_i are therefore lower limits for the interaction distance under conditions of small internal load.

The effect of the reduced [ATP] and accumulating hydrolysis products toward the end of shortening can be estimated as follows: when the ATP available is reduced and ADP and inorganic phosphate (P_i) are added to the medium, the conditions simulate the later phase of shortening from higher starting [ATP]. If D_i obtained at $370 \mu\text{M}$ $[\text{ATP}_u]$ plus $300 \mu\text{M}$ initial [ADP] and $[P_i]$ (22 nm; triangle in Fig. 3) corresponds to the final phases of ATP hydrolysis from $688 \mu\text{M}$ $[\text{ATP}_u]$ (40 nm, open circle at low load in Fig. 3), the D_i in the initial phase between 688 and $370 \mu\text{M}$ ATP is given by $(40 \times 688 - 22 \times 370)/(688 - 370) = 61 \text{ nm}$. Even 61 nm is a lower limit for the interaction distance at low load because shortening velocities were not saturated at the experimental $[\text{ATP}_u]$ and actomyosin rigor complexes pose an internal load.

In Huxley's model of the actomyosin crossbridge cycle⁸, crossbridges are postulated to generate positive forces and also to

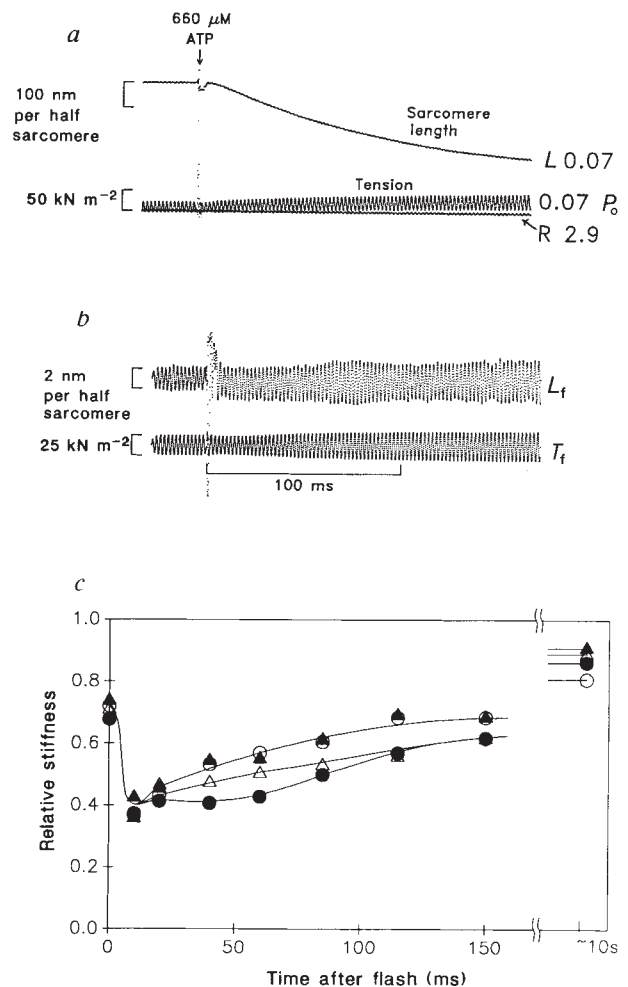


FIG. 2 Stiffness measurements during isotonic shortening of skinned fibres. *a*, Sarcomere length (L 0.07), tension ($0.07 P_0$) and base line (R 2.9) traces from the same experiment shown in Fig. 1a. The data are displayed without filtering and on a faster time base. The sarcomere-length signal was electronically gated off during the photolysis laser pulse. *b*, Sarcomere length (L_f) and tension (T_f) filtered through 500 Hz, $Q = 2$, recursive digital bandpass filters to isolate the sinusoidal components. Stiffness was measured as the ratio of the amplitude of T_f to that of L_f . *c*, Stiffness (S_r) following photorelease of $530\text{--}713 \mu\text{M}$ ATP relative to the stiffness in rigor at full overlap. Each plotted point shows the mean of four to eight measurements in isometric contractions ($\circ$), or during shortening at $0.47 \pm 0.04 P_0$ ($n = 4$, $\blacktriangle$), $0.25 \pm 0.01 P_0$ ($n = 4$, $\triangle$) or $0.07 \pm 0.01 P_0$ ($n = 8$, $\bullet$). The lines were drawn by eye.

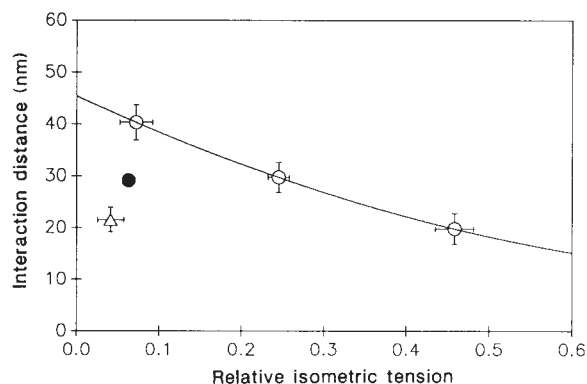


FIG. 3 Calculated sliding distance (D_i) of myosin heads interacting with thin filaments per ATP molecule hydrolysed. $\circ$, From experiments with hydrolysed $[ATP_0] > 500 \mu M$ including the data shown in Fig. 2; $[ATP_0] = 688 \pm 40 \mu M$ (s.e.m., $n=12$), $807 \pm 89 \mu M$ ($n=8$) and $791 \pm 116 \mu M$ ($n=7$) at 0.07, 0.25 and 0.46 P_0 , respectively. The curve drawn through the open circles is a parabola. $\bullet$, $[ATP_0] = 336 \pm 15 \mu M$ ($n=13$). Δ , $[ATP_0] = 370 \pm 2 \mu M$ ($n=5$); 300 μM ADP and 300 μM P_i were added to the medium. Means $\pm$ s.e.m. are plotted for 5–17 fibres; s.e.m. for the closed circle was within the symbol.

bear negative drag force. The force generated by a muscle during shortening is the difference between these working and drag forces. The interaction distance per ATP molecule measured in our experiments is the sum of the working and drag distances. If the length of the working stroke does not depend on velocity, then our result that D_i increases with velocity implies that the drag distance increases with velocity as predicted by Huxley's model.

The 40 nm or 61 nm lower limits for D_i at low loads indicate that myosin and actin interact over a considerably greater sliding distance than a single tilting motion of the head, which might translate the filaments by about 12 nm (refs 1, 3–5). The large D_i can be incorporated into the cross-bridge cycle in several ways. If the power stroke is 12 nm and negatively strained crossbridges are less stiff than working crossbridges, the remaining ≥ 28 nm or ≥ 49 nm of D_i might be drag distance. The structure of crossbridges negatively strained by ≥ 28 nm should be recognizable in electron micrographs. Alternatively, for each ATPase cycle at high velocities, myosin heads might interact mechanically with several actin monomers either at positive or negative strain, as suggested by other recent studies^{7,9,17–19}. $\square$

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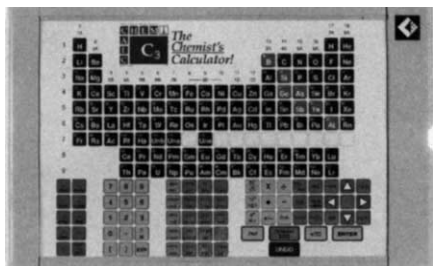
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Scientific shopping spree

Assorted products featured this week include a chemist's calculator, a brain infusion kit, an all-in-one stirrer and balance, and gel chambers with built-in buffer recirculation.

CHEMiCALC, a **calculator for chemists** that is available from Chemical Concepts, is designed to perform quickly and accurately many of the calculations routinely carried out by chemists (*Reader Service No. 100*). Simple mathematical errors are eliminated, so that productive time is spent on the problem set-up and unit conversion explorations, says Bert Ramsay, inventor of the new product. Explorations are facilitated by a calculation UNDO key. The intuitive approach to chemical calculations is enhanced by a touchpad that includes a periodic table of the elements, in addition to the standard calculator keys. With the CHEMiCALC touchpad and software, six calculation



CHEMiCALC keypad.

modes are available, including chemical formula weight calculations obtained from the chemical formulas 'written' from the periodic table touchpad. Unit conversions and other chemical calculation problem set-ups can be explored and corrected in the CALCULATION mode by examining the displayed set-ups and results. Limiting reagent calculations and explorations are performed quickly in the REACTION mode. Empirical formula determinations, from entered analytical data, routinely are completed in less than a minute, Ramsay says. A hand-held or notebook calculator is planned for release in mid-1992. The standard Version 1.5 of CHEMiCALC for IBM PCs or compatibles is offered until September at an introductory price of \$349 (US).

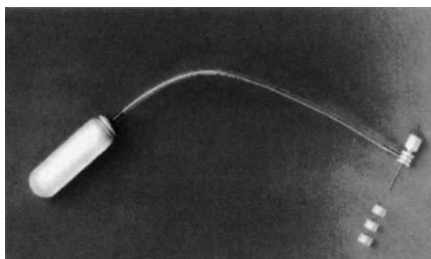
Jencons Scientific has introduced two battery-powered **equipment alarms**, the Fly and the Spider, which are designed to protect expensive items of equipment in the laboratory from theft (*Reader Service No. 101*). The Fly can be used to protect single items. A security key activates/deactivates the alarm once it is attached to the instrument. In the event of the instrument being moved, an alarm sounds, which lasts for 2–2.5 hours. Working on the same principle as the Fly, the Spider



Alarming new ideas from Jencons.

can protect up to five instruments with its arrangement of tentacles. If the tentacles are disturbed in any way, the alarm sounds.

Alza announces the availability of its new ALZET Brain Infusion Kit, which targets the **delivery of test compounds to the central nervous system of laboratory rats** for extended periods of time (*Reader Service No. 102*). The kit is designed specifically for use with the company's osmotic pumps, which are miniature implantable infusion systems used to determine the effects of controlled substance delivery. The kit features an adjustable



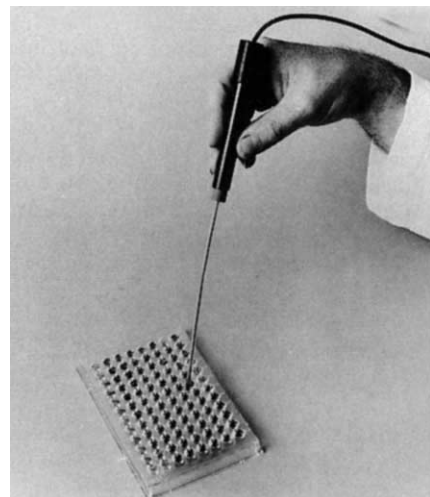
Probing the brain — see Alza.

cannula, which allows researchers to alter the depth of the brain infusion site. Using the depth-adjustment spacers that are supplied with the kit, it is possible to optimize infusion depth for a particular structure, target different brain regions, or make adjustments for animal size. The kit provides a means of bypassing the blood-brain barrier, allowing experimentally useful quantities of candidate

agents to be infused directly into the brain, Alza says. Each \$40 (US) Brain Infusion Kit contains sufficient materials for ten brain infusions: ten 28-gauge, L-shaped, stainless steel cannulae, ten catheter tubes, 40 depth-adjustment spacers and a set of instructions.

Handy gadgets

Small is beautiful if you need to measure pH in liquid samples of no more than 10 μ l. The PHR-146 **micro pH electrode** from Lazar Research Laboratories, which has an active element measuring just 1 mm, is designed to do just that (*Reader Service*



Making pH measurements on a micro scale.

No. 103). The pH microprobe can be used in a variety of receptacles, such as microtitre plates, culture tubes, cuvettes, microtubes, serum cups and nuclear magnetic resonance cups. The probe's flat sensor tip can be used to provide measurements on surfaces such as filter paper and agar surface gels.

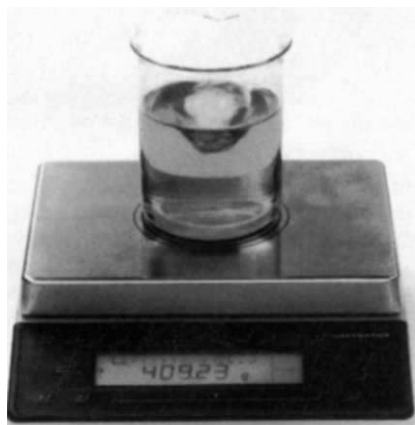
Eliminate the need to make routine trips to the ice-making machine with the new BioChiller thermoelectrically-controlled **benchtop cooling devices** from Fotodyne (*Reader Service No. 104*). Two models are available: the BioChiller 1000, which cools samples to 2 °C, and the BioChiller 2000, which provides temperature control from –5 to 25 °C. In both units the samples are held securely in the wells of an aluminium cooling block. In addition, both units are fitted with recessed handles, a hinged lid for convenient viewing, and one of four cooling blocks to accommodate 0.65-ml microtubes, 1.7-ml microtubes, a combination



Chill out with Fotodyne's benchtop cooling devices.

of 0.65-ml and 1.7-ml microtubes, or 12-mm diameter test tubes. Additional blocks can be purchased separately. The BioChiller 2000 continuously measures the temperature of the cooling block and displays it on a three-digit LED. Fotodyne says these benchtop cooling devices, which occupy about the same amount of space as an ice bucket, can be used for the temporary storage of enzymes and samples that are required for minipreps, TCA precipitations, ligation reactions and timed reactions. Prices for the BioChiller 1000 and 2000 models start at \$975 (US) and \$1,250 (US), respectively.

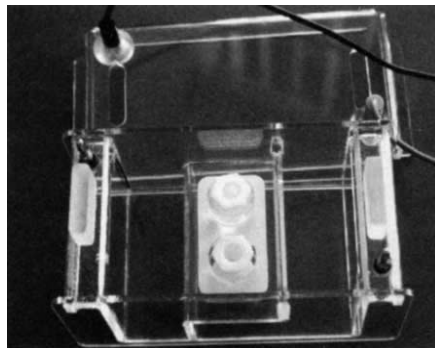
Combining two devices in one, the new **magnetic stirrer balance** from Sartorius offers users the capability of stirring liquids and weighing accurately down to 0.01 g, all at the same time (*Reader Service No. 105*). With the magnetic stirrer balance, the stirrer is integrated into the weighing pan and is operated using the control panel on the balance. The balance



Sartorius offers two devices in one with the combination stirrer and balance.

has a weighing capacity of 4,200 g, which is accurate to 0.01 g, Sartorius says. The instrument's weighing and stirring functions can also be used independently. Effective shielding from magnetic fields and signal filtering by MC1 technology ensure that the weight readout is not affected by the magnetic field of the stirrer, or by the motion of a liquid as it is being stirred. The stirrer and balance can also be hooked up, via the RS232 interface, to automated laboratory systems.

Genzomed has introduced an **electroelution device** for the elution of proteins and nucleic acids from polyacrylamide and agarose gels, PVDF and nitrocellulose membranes (*Reader Service No. 106*). The sample is collected in a chamber, which is made of Teflon to facilitate high recovery, with a predetermined volume (10–1,500 µl). The sample, without further handling, is then dialysed against a desired solution to remove salts and other molecules. The entire chamber can be put under vacuum and concentrated up to the required volume. The



Genzomed's complete electroelution kit.

\$599 (US) ready-to-use electroelution unit includes the elution tank, elution chamber and a choice of pre-cut membranes with molecular weight cut-offs ranging from 100 to 500,000 daltons. Two, four or more samples can be eluted simultaneously.

In kit form

Baxter has introduced a new kit that uses self-sustained sequence replication, or 3SR, a **nucleic acid amplification technology** that does not require the use of a thermal cycler and which is designed to produce at least 10^6 -fold amplification from 10^{-20} moles of specific RNA in one hour (*Reader Service No. 107*). The reactions involved in self-sustained sequence replication are run in a single tube isothermally. The technology is based on a retroviral strategy of RNA replication by oligonucleotide priming and enzymatic formation of cDNA intermediates. The predominant end products of the reaction are a mixture of specific RNA and cDNA, present in a ratio of 100 to 1, respectively, Baxter says. Unlike PCR, which requires the conversion of all RNA in the sample to cDNA before the annealing of specific primers, 3SR anneals specific primers to the target RNA in the sample and copies that template to cDNA with reverse transcriptase. Amplification is then accomplished by the generation of RNA from double-stranded cDNA. The technology, which can be used to amplify specific sequences of RNA that are present in extremely small amounts in cells, has applications in the study of viruses and genetic diseases, and can be used as a tool for preparing specific sense and antisense RNA. Each 3SR kit includes sufficient

buffer, nucleotides and enzymes (reverse transcriptase, T7 RNA polymerase, RNase H) for 100 reactions, although a 50-reaction size kit is also available.

All you require to **determine the presence and approximate number of N-glycans in a glycoprotein** by band-shift electrophoresis in the SDS-PAGE is provided in the new Protein de-N - Glycosylation Kit from Oxford GlycoSystems (*Reader Service No. 108*). The kit can also be used to estimate the molecular weight of a polypeptide backbone after removal of covalently attached glycans. The kit contains a highly purified and highly characterized preparation of PNGase F, which is free of protease and endoglycosidase activity, Oxford GlycoSystems says, together with a protocol for denaturation of sample glycoprotein and its subsequent incubation with PNGase F. To identify the number of N-glycans attached to a polypeptide, first complete hydrolysis and then a time-course of hydrolysis by PNGase F are performed, the latter is carried out to achieve partial de-N-glycosylation. The number of bands on SDS-PAGE between untreated and completely hydrolysed glycoprotein indicates the approximate number of N-glycans on the original glycoprotein, the company says. Oxford GlycoSystems notes, however, that this method is useful only when the accuracy of the SDS-PAGE system allows changes in protein mobility of ≥ 3 kD to be detected, which may be difficult for proteins of very high molecular weight or low mobility. The kit includes sufficient reagents and controls for the analysis of ten samples and two sets of control glycoproteins of established susceptibility to PNGase F.

Pure and simple

Sterogene Bioseparations announces the availability of its **QUICKMab monoclonal antibody purification system** (*Reader Service No. 109*). QUICKMab provides a quick and easy way to purify, desalt and concentrate monoclonal antibodies from cell culture or ascitic fluid without the need for methods development, Sterogene says. The kit can be used to purify any class or subclass of antibody, including IgG, IgM, IgA and IgE, to greater than 98 per cent purity in about an hour. QUICKMab purifies antibodies at neutral pH under protein stabilizing conditions. If serum supplements are used in the culture medium, the company says the pure antibody will be free of bovine IgG contaminants: no dialysis or concentration steps are required.

PROSEP-A, which consists of **Protein A immobilized onto a novel support matrix** called PROSEP, is now available in pre-packed columns from Bioprocessing (*Reader Service No. 110*). The columns are

suitable for analytical or semi-preparative antibody purification direct from cell culture supernatant, ascites or serum/plasma. Because of its physical properties, PROSEP-A can withstand very high flow rates, Bioprocessing says. Typically, flow rates of 100 times column volume per hour will result in greater than 98 per cent recovery of a mouse IgG₁ whose initial concentration in the cell culture filtrate is 10 µg ml⁻¹ or greater. The prepacked columns are available in 2-ml, 10-ml or 25-ml sizes and are supplied with all the accessories that are required for the binding of IgG from a variety of feedstocks. The accessories provide for column loading from a syringe, peristaltic pump or FPLC system. The company claims that the binding capacity of PROSEP-A for IgG₁ from all species is much higher than that typically exhibited by other conventional Protein-A adsorbents, particularly with mouse IgG₁, where high pH and high salt concentrations are often necessary to increase binding capacity even though this can lead to increased non-specific binding. With PROSEP-A, antibodies can be bound efficiently under physiological conditions, the company says.

Transfection tools

The Porator from Invitrogen is a versatile capacitance discharge unit that is designed for reliable and efficient **electroporation of *Escherichia coli*, mammalian and yeast cells** (Reader Service No. 111). The company claims that both large and small DNA molecules are electroporated at high efficiencies, which are linear over a wide range of DNA concentrations. A range of settings are available, allowing electroporation of a wide variety of cells. The Porator increases the transformation efficiency of bacterial cells by greater than 100 fold over chemical transformation methods, says Invitrogen, which allows the production of increased cDNA library sizes. Each unit has easy-to-read displays for load resistance and capacitance settings and indicators for both charging and discharging. The \$795 (US) Porator is supplied with an instruction manual, five vials of electro-competent bacterial cells and ten 0.1-cm gap cuvettes. The unit is compatible with most electrophoresis and sequencing power supplies.

Cellject is the new **electroporation system** from Flowgen for the transfection of mammalian, plant, yeast and bacterial cells (Reader Service No. 112). The system uses a patented double-pulsed system that is designed to reduce cellular shock and cell mortality and increase transformation efficiencies. All programmed and measured parameters are monitored constantly. Hard-copy records of these parameters can be obtained by linking the Cellject up to a printer via its serial printer

port. Safety features of Cellject include the detection of arc, open and short circuits.

Benchtop aids

NovaSyn Crystal is a new generation of **peptide synthesizer** from Novabiochem that features feedback control of all aspects of synthesis (Reader Service No. 113). The CDM acylation monitoring allows the control software to extend automatically acylation and deprotection times, where necessary, resulting in purer peptides and avoiding costly sequence failures, Novabiochem says. The mouse-driven software is supplied with a range of standard protocols, including PyBOP/HBTU and active ester coupling, with the facility to add user-defined protocols when required. The instrument is backed up by a broad range of prepacked reagents, which offer two synthesis scales for ODhbt and OPfp esters and the convenience of premixed Fmoc amino acids and PyBOP for fast, efficient coupling, the company says.

Fisher Scientific has introduced a new **gel dryer**, which has been designed to eliminate the problems of distortion and cracking and so provide high-quality electrophoresis gels for autoradiography, densitometry, photography or storage in a notebook (Reader Service No. 114). The



High and dry — see Fisher.

Teflon-coated aluminium casting provides an even distribution of heat and so eliminates the formation of 'hot spots', which can cause cracking, Fisher says. The temperature of the unit can be adjusted from ambient to 80°C. The \$1,125 (US) gel dryer provides a 37×47 cm drying surface, which is sufficient to accommodate most gel sizes from multiple minigels to large DNA sequencing gels.

Gel boxes

Hoefer has developed a new **horizontal submarine electrophoresis unit**, the HE 120 SimpleSub, which features internal buffer recirculation (Reader Service No. 115). To prevent ion depletion and changes in pH, buffer is recirculated internally over the surface of the gel. This, Hoefer says, eliminates the expense of having to provide an external pump and prevents the risk of accidental contact with charged buffer. The HE 120 can accommodate large gel sizes of 20-cm

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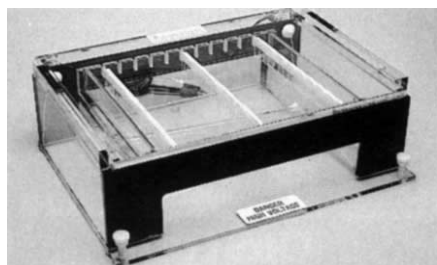
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wide by 15-, 20- or 25-cm long, which allows a large number of samples to be electrophoresed at any one time. To drive the buffer circulation impeller, the HE 120 can be used with the purpose-built MS 100 SpinStir wide platform. Stated applications of the HE 120 include RNA electrophoresis, RFLP analysis and DNA fingerprinting studies.

The Puffer Buffer is the new **self-recirculating electrophoresis chamber** from Owl Scientific that combines the features of the company's horizontal gel systems with a patented buffer recirculation system (*Reader Service No. 116*). The instrument has been designed to collect the hydrogen bubbles generated at the cathode, and use this flow to pump buffer through an internal conduit from one chamber to the other: no external pumps, tubing, magnetic stir bars are necessary, Owl says. This set-up ensures uniform ionic strength throughout the system, and eliminates the problem of uneven migration and banding patterns. Two sizes of chamber are available: a \$425 (US) standard system (20×25 cm gel size) and a \$295 (US) minigel system (12×14 cm gel



Puffer Buffer — horizontal gel box with built-in buffer recirculation.

size). Each system includes gasketed end gates for no-tape gel casting, a three-point levelling system, a UV-transmissible gel tray, three combs, a SuperSafe interlock lid with attached power cords, platinum electrodes, and no-skid rubber feet.

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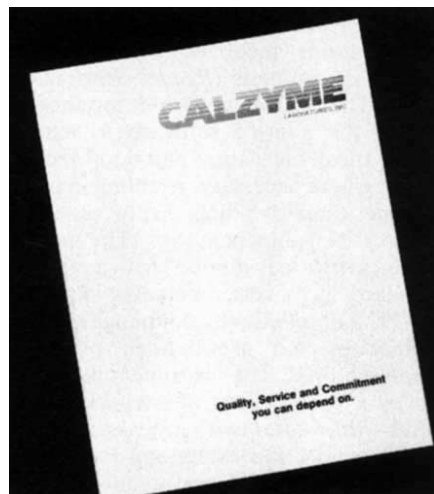
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Reagent round-up

Over 200 products are listed in the 1992 **catalogue of enzymes, proteins, substrates** and related biochemicals from Calzyme Laboratories (*Reader Service No. 117*).



Calzyme's buyer's guide.

New additions include a highly specific alkaline phosphatase and peroxidase for enzyme-antibody conjugation. Although the products are available in research quantities, Calzyme can supply bulk quantities.

A broad range of growth factors, growth factor receptors and monoclonal and polyclonal antibodies for **growth factor and receptor research** are available from Austral Biologicals (*Reader Service No. 118*). Austral's line up of growth factors includes EGF, IGF I, IGF II, PDGF AA, PDGF BB, NGF, TNF and FGF. The recombinant growth factor receptors include human EGF receptor (extracellular domain), human NGF receptor (extracellular domain), human FGF receptor (extracellular domain) and human PDGF receptor (extracellular domain). The company also supplies a complete line of polyclonal and monoclonal antibodies for growth factor and receptor research.

Chromatography corner

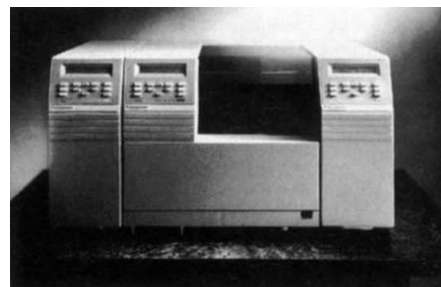
Ciba Corning is distributing Jasco's Model 851-AS microcomputer-based **HPLC autosampler**, which has a 100-sample capacity (*Reader Service No. 119*). Both variable-volume and fixed-loop injection are offered as standard with the Model 851-AS. When in variable-volume mode, injections of 1–200 μ l in 1- μ l increments can be made; 201–1,000- μ l injections are an optional extra. The standard fixed-loop volume is 100 μ l; 10-, 20-, 50-, 200-, 500- and 1,000- μ l loops are optional. The autosampler provides less than $\pm 0.5\%$ reproducibility in both variable-volume (for 100- μ l injections) and fixed-loop injection modes, the company says. Other design features include automatic pre-



Automated sample injection system.

column derivatization and X-Y-Z axis needle arm control for random sample access and adjustable needle height to allow sampling from the top or bottom of a vial. To preserve biological material and ensure reproducible injections of even highly volatile samples, a built-in refrigeration unit is offered as an optional extra. Prices for the Model 851-AS, which can be interfaced with most chromatography systems, start at around \$7,500 (US).

With flexibility in mind, Spectra-Physics has introduced a new modular line of **instrumentation for liquid chromatography** called SpectraSYSTEM, which



Mix and match with the SpectraSYSTEM.

consists of a selection of pumps, autosamplers and detectors that can be configured to meet specific needs (*Reader Service No. 120*). This is facilitated because SpectraSYSTEM instruments share many common features: all models have a common keypad design and display. Spectra-Physics offers a selection of pumps, including isocratic, binary isocratic, and binary and quaternary models. Most of the 120-sample capacity autosamplers provide on-line sample preparation for heated derivatizations, extractions and dilutions. A choice of fluorescence and single- or dual-wavelength UV/visible detectors is provided. In addition, all instruments are available in inert/biocompatible versions. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quotes are sometimes nominal, and apply only within the country indicated.